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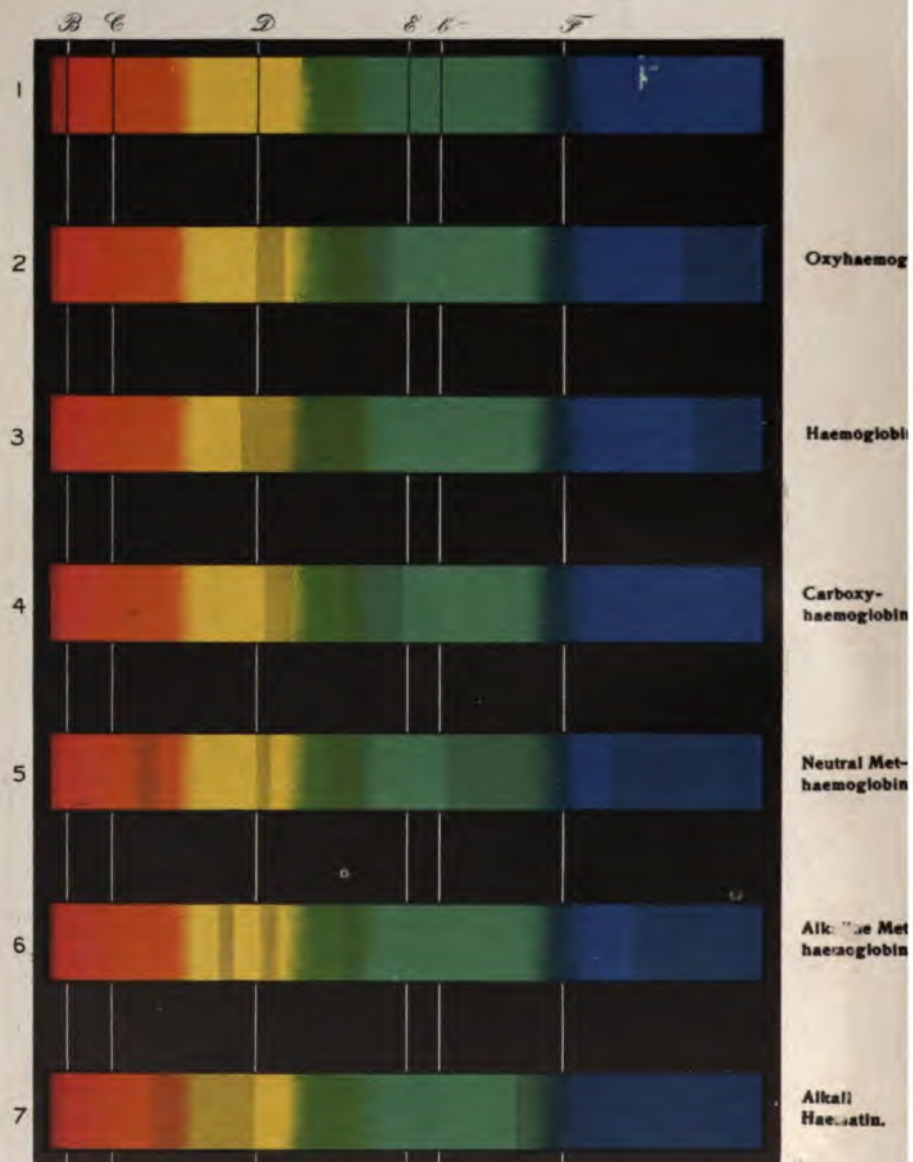


PRACTICAL
PHYSIOLOGICAL CHEMISTRY

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HAWK

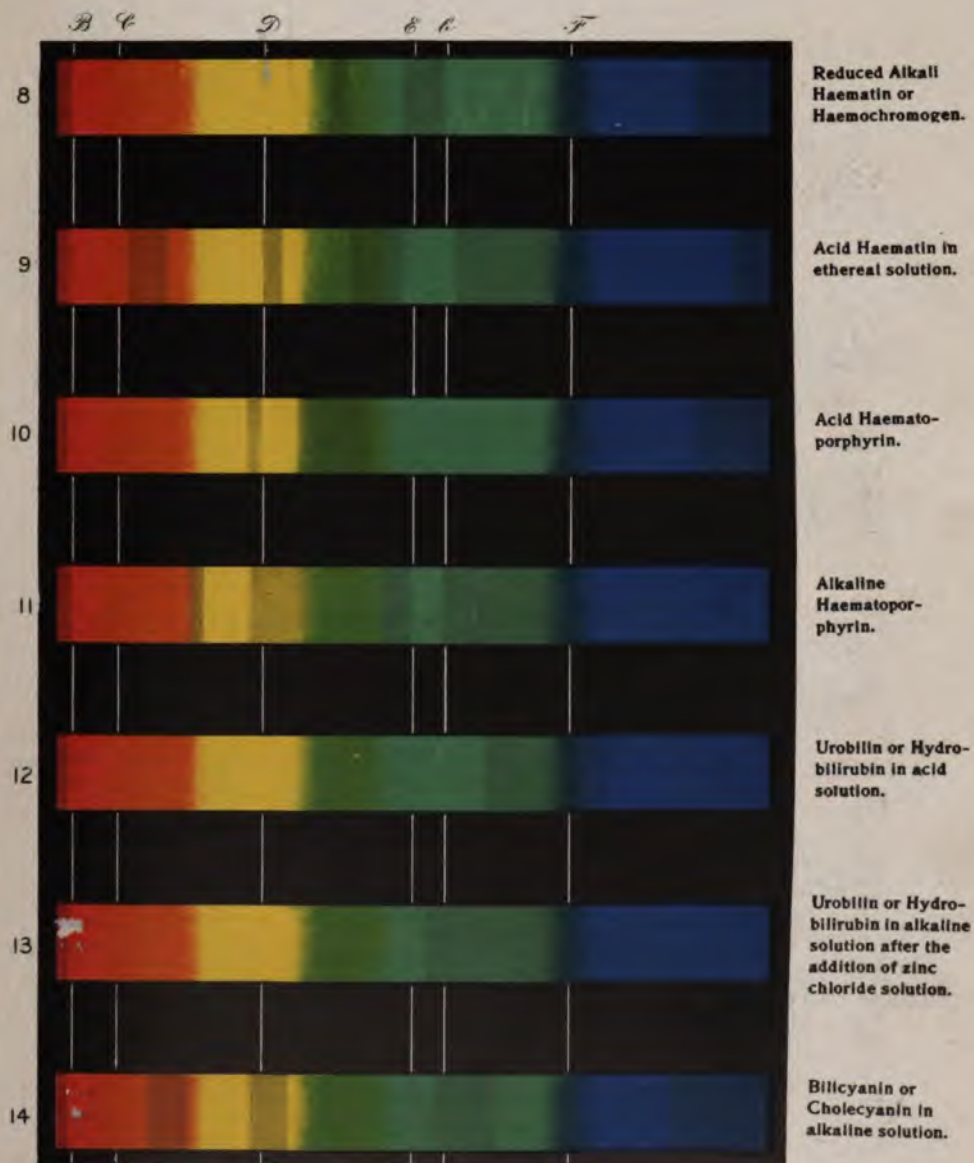
Absorption Spectra.

PLATE I.



Absorption Spectra.

PLATE II.



PRACTICAL PHYSIOLOGICAL CHEMISTRY

A BOOK DESIGNED FOR USE IN COURSES
IN PRACTICAL PHYSIOLOGICAL CHEMISTRY
IN SCHOOLS OF MEDICINE AND OF SCIENCE

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PHILIP B. HAYES, M.S., Ph.D.

DEMONSTRATOR OF PHYSIOLOGICAL CHEMISTRY IN THE DEPARTMENT OF MEDICINE
OF THE UNIVERSITY OF PENNSYLVANIA

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THESE PAGES
ARE AFFECTIONATELY DEDICATED
TO
MY MOTHER

PREFACE

The plan followed in the presentation of the subject of this volume is rather different, so far as the author is aware, from that set forth in any similar volume. This plan, however, he feels to be a logical one and has followed it with satisfactory results during a period of three years in his own classes at the University of Pennsylvania. The main point in which the plan of the author differs from those previously proposed is in the treatment of the food stuffs and their digestion.

In Chapter IV the "Decomposition Products of Proteids" has been treated although it is impracticable to include the study of this topic in the ordinary course in practical physiological chemistry. For the specimens of the decomposition products, the crystalline forms of which are reproduced by original drawings or by micro-photographs, the author is indebted to Dr. Thomas B. Osborne, of New Haven, Conn.

Because of the increasing importance attached to the examination of feces for purposes of diagnosis, the author has devoted a chapter to this subject. He feels that a careful study of this topic deserves to be included in the courses in practical physiological chemistry, of medical schools in particular. The subject of *solid tissues* (Chapters XIII, XIV and XV) has also been somewhat more fully treated than has generally been customary in books of this character.

The author is deeply indebted to Professor Lafayette B. Mendel, of Yale University, for his careful criticism of the manuscript and to Professor John Marshall, of the University of Pennsylvania, for his painstaking revision of the proof. He also wishes to express his gratitude to Dr. David L. Edsall for his criticism of the clinical portion of the volume; to Dr. Otto Folin for suggestions regarding several of his quantitative methods, and to Mr. John T. Thomson for assistance in proof-reading.

For the micro-photographs of oxyhæmoglobin and hæmin reproduced in Chapter XI the author is indebted to Professor E. T. Reichert, of the University of Pennsylvania, who, in collaboration with Professor A. P. Brown, of the University of Pennsylvania, is making a very extended investigation into the crystalline forms of biochemic substances. The micro-photograph of allantoin was kindly furnished by Professor Mendel. The author is also indebted for suggestions and assistance received from the lectures and published writings of numerous authors and investigators.

The original drawings of the volume were made by Mr. Louis Schmidt whose eminently satisfactory efforts are highly appreciated by the author.

PHILIP B. HAWK.

PHILADELPHIA, March 27, 1907.

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PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

CARBOHYDRATES.

THE name carbohydrates is given to a class of bodies which are an especially prominent constituent of plants and which are found also in the animal body either free or as an integral part of various proteids. They are called carbohydrates because they contain the elements C, H and O; the H and O being present in the proportion to form water. The term is not strictly appropriate inasmuch as there are bodies such as acetic acid, lactic acid and inosit which have H and O present in the proportion to form water, but which are not carbohydrates, and there are also true carbohydrates which do not have H and O present in this proportion, e. g., rhamnose, $C_6H_{12}O_5$.

Chemically considered, the carbohydrates are aldehyde or ketone derivatives of complex alcohols. Treated from this standpoint the aldehyde derivatives are spoken of as aldoses, and the ketone derivatives are spoken of as ketoses. The carbohydrates are also frequently named according to the number of carbon atoms present in the molecule, e. g., trioses, pentoses and hexoses.

The more common carbohydrates may be classified as follows:

I. Monosaccharides.

1. Hexoses, $C_6H_{12}O_6$.

(a) Dextrose.

(b) Lævulose.

- (c) Galactose.
- 2. Pentoses, $C_5H_{10}O_5$.
 - (a) Arabinose.
 - (b) Xylose.
 - (c) Rhamnose (Methyl-pentose), $C_6H_{12}O_5$.
- II. Disaccharides, $C_{12}H_{22}O_{11}$.
 - 1. Maltose.
 - 2. Saccharose.
 - 3. Iso-Maltose.
 - 4. Lactose.
- III. Trisaccharides, $C_{18}H_{32}O_{16}$.
 - 1. Raffinose.
- IV. Polysaccharides, $(C_6H_{10}O_5)_x$.
 - 1. Starch Group.
 - (a) Starch.
 - (b) Inulin.
 - (c) Glycogen.
 - (d) Lichenin.
 - 2. Gums and Vegetable Mucilage Group.
 - (a) Dextrin.
 - (b) Vegetable Gums.
 - 3. Cellulose Group.
 - (a) Cellulose.
 - (b) Hemi-Cellulose.

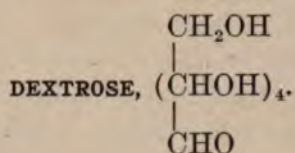
Each member of the above carbohydrate classes, except the members of the pentose group, may be supposed to contain the group $C_6H_{10}O_5$ called the *saccharide group*. The polysaccharides consist of this group alone taken a large number of times, whereas the disaccharides may be supposed to contain two such groups plus a molecule of water, and the monosaccharides to contain one such group plus a molecule of water. Thus, $(C_6H_{10}O_5)_x = \text{polysaccharide}$, $(C_6H_{10}O_5)_2 + H_2O = \text{disaccharide}$, $C_6H_{10}O_5 + H_2O = \text{monosaccharide}$. In a general way the solubility of the carbohydrates varies with the number of saccharide groups present, the substances containing the largest number of these groups being the least

soluble. This means simply that, as a class, the monosaccharides (hexoses) are the most soluble and the polysaccharides (starches and cellulose) are the least soluble.

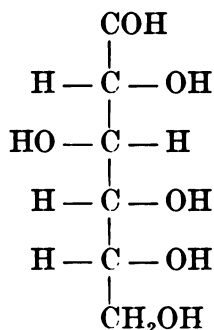
MONOSACCHARIDES.

Hexoses, $C_6H_{12}O_6$.

The hexoses are monosaccharides containing six carbon atoms to the molecule. They are the most important of the simple sugars, and two of the principal hexoses, dextrose and lævulose, occur widely distributed in plants and fruits. These two hexoses also result from the hydrolysis of starch and cane sugar. Galactose, which with dextrose results from the hydrolysis of lactose, is also an important hexose. These three hexoses are fermentable by yeast, and yield lævulinic acid upon heating with dilute mineral acids. They reduce metallic oxides in alkaline solution, are optically active, and extremely soluble. With phenylhydrazin they form characteristic osazons.



Dextrose, also called glucose, grape sugar, or diabetic sugar, is present in the blood in small amount and may also occur in traces in normal urine. After the ingestion of large amounts of saccharose, lactose or dextrose an alimentary glycosuria occasionally arises. In diabetes mellitus very large amounts of dextrose are excreted in the urine. The following structural formula has been suggested by Victor Meyer for *d*-dextrose:

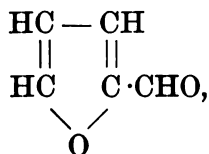


(For further discussion of dextrose see section on Hexoses, page 3.)

EXPERIMENTS ON DEXTROSE.

1. **Solubility.**—Test the solubility of dextrose in the “ordinary solvents” and in alcohol. (In the solubility tests throughout the book we shall designate the following solvents as the “ordinary solvents”: H_2O ; 10 per cent NaCl ; 0.5 per cent Na_2CO_3 ; 0.2 per cent HCl ; concentrated KOH ; concentrated HCl .)

2. **Molisch's Reaction.**—Place approximately 5 c.c. of concentrated H_2SO_4 in a test-tube. Incline the tube and slowly pour down the inner side of it approximately 5 c.c. of the sugar solution to which 2 drops of α -naphthol solution (about 15 per cent alcoholic solution) has been added, so that the sugar solution will not mix with the acid. A reddish-violet zone is produced at the point of contact. The reaction is due to the formation of furfurol,



by the acid. The test is given by all bodies containing a carbohydrate group and is therefore of very little practical importance.

PLATE III.

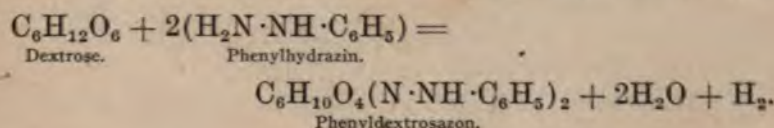


OSAZONS.

Upper form, dextrosazon; central form, maltosazon; lower form, lactosazon.

3. **Phenylhydrazin Reaction.**—Test according to one of the following methods: (a) To a small amount of phenylhydrazin mixture, furnished by the instructor,¹ add 5 c.c. of the sugar solution, shake well and heat on a boiling water-bath for one-half to three-quarters of an hour. Allow the tube to cool *slowly* and examine the crystals microscopically (Plate III, opposite). If the solution has become too concentrated in the boiling process it will be light-red in color and no crystals will separate until it is diluted with water.

Yellow crystalline bodies called *osazons* are formed from certain sugars under these conditions, each individual sugar giving rise to an osazon of a definite crystalline form which is typical for that sugar. Each osazon has a definite melting-point and as a further and more accurate means of identification it may be recrystallized and identified by the determination of its melting-point and nitrogen content. The reaction taking place in the formation of *phenyldextrosazon* is as follows:



(b) Place 5 c.c. of the sugar solution in a test-tube, add 1 c.c. of the phenylhydrazin-acetate solution furnished by the instructor,² and heat on a boiling water-bath for one-half to three-quarters of an hour. Allow the liquid to cool *slowly* and examine the crystals microscopically (Plate III, opposite).

The phenylhydrazin test has been so modified by Cipollina as to be of use as a *rapid clinical test*. The directions for this test are given in the next experiment.

¹ This mixture is prepared by combining one part of phenylhydrazin-hydrochloride and two parts of sodium acetate, *by weight*. These are thoroughly mixed in a mortar.

² This solution is prepared by mixing one part *by volume*, in each case, of glacial acetic acid, one part of water and two parts of phenylhydrazin (the base).

✓ *omit* 4. **Cipollina's Test.**—Thoroughly mix 4 c.c. of dextrose solution, 5 drops of phenylhydrazin (the base) and $\frac{1}{2}$ c.c. of glacial acetic acid in a test-tube. Heat the mixture for about one minute over a low flame, shaking the tube continually to prevent loss of fluid by bumping. Add 4–5 drops of sodium hydroxide (sp. gr. 1.16), being certain that the fluid in the test-tube remains acid, heat the mixture again for a moment and then cool the contents of the tube. Ordinarily the crystals form at once, especially if the sugar solution possesses a low specific gravity. If they do not appear immediately allow the tube to stand at least 20 minutes before deciding upon the absence of sugar.

Examine the crystals under the microscope and compare them with those shown in Plate III, opposite page 5.

✓ 5. **Precipitation by Alcohol.**—To 10 c.c. of 95 per cent alcohol add about 2 c.c. of dextrose solution. Compare the result with that obtained under Dextrin, 7, page 28.

6. **Iodine Test.**—Make the regular iodine test as given under Starch, 5, page 24, and compare this result with the results obtained with starch and with dextrin.

omit 7. **Diffusibility of Dextrose.**—Test the diffusibility of dextrose solution through animal membrane, or parchment paper,

FIG. 1.

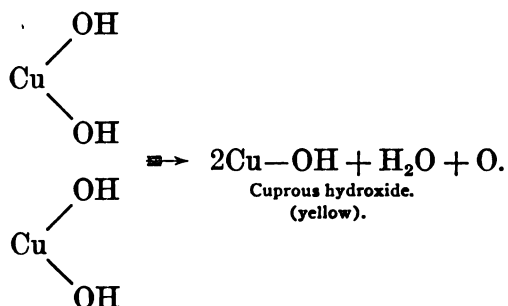
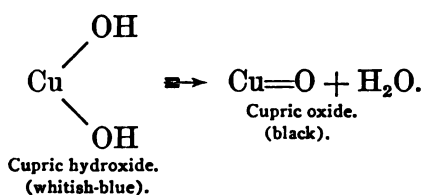


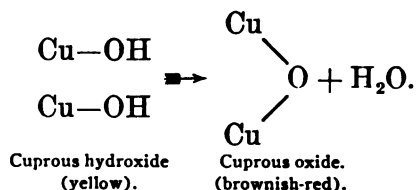
DIALYZING APPARATUS FOR STUDENTS' USE.

making a dialyzer like one of the models shown in Fig. 1, above.

8. **Moore's Test.**—To 2–3 c.c. of sugar solution in a test-tube add an equal volume of concentrated KOH or NaOH, and boil. The solution darkens and finally assumes a brown color. This is an exceedingly crude test and is of little practical value.

9. **Reduction Tests.**—To their aldehyde or ketone structure many sugars owe the property of readily reducing alkaline solutions of the oxides of metals like copper, bismuth and mercury; they also possess the property of reducing ammoniacal silver solutions with the separation of metallic silver. Upon this property of reduction the most widely used tests for sugars are based. When whitish-blue cupric hydroxide in suspension in an alkaline liquid is heated it is converted into insoluble black cupric oxide, but if a reducing agent like certain sugars be present the cupric hydroxide is reduced to insoluble yellow cuprous hydroxide, which in turn on further heating may be converted into brownish-red or red cuprous oxide. These changes are indicated as follows:





The chemical equations here discussed are exemplified in Trommer's and Fehling's tests.

✓ (a) *Trommer's Test*.—To 5 c.c. of sugar solution in a test-tube add one-half its volume of KOH or NaOH. Mix thoroughly and add, drop by drop, a *very dilute* solution of cupric sulphate. Continue the addition until there is a slight permanent precipitate of cupric hydroxide and in consequence the solution is slightly turbid. Heat, and the cupric hydroxide is reduced to yellow cuprous hydroxide or to brownish-red cuprous oxide. If the solution of cupric sulphate used is too strong a small brownish-red precipitate produced in a weak sugar solution may be entirely masked. On the other hand, particularly in testing for sugar in the urine, if too little cupric sulphate is used a light-colored precipitate formed by uric acid and purin bases may obscure the brownish-red precipitate of cuprous oxide. The action of KOH or NaOH in the presence of an excess of sugar and insufficient copper will produce a brownish color. Phosphates of the alkaline earths may also be precipitated in the alkaline solution and be mistaken for cuprous hydroxide. Trommer's test is not very satisfactory.

✓ (b) *Fehling's Test*.—To about 1 c.c. of Fehling's solution¹

¹ Fehling's solution is composed of two definite solutions—a cupric sulphate solution and an alkaline tartrate solution, which may be prepared as follows:

Cupric sulphate solution = 34.64 grams of cupric sulphate dissolved in water and made up to 500 c.c.

Alkaline tartrate solution = 125 grams of potassium hydroxide and 173 grams of Rochelle salt dissolved in water and made up to 500 c.c.

These solutions should be preserved separately in rubber-stoppered bottles and mixed in equal volumes when needed for use. This is done to prevent deterioration.

in a test-tube add about 4 c.c. of water, and boil. This is done to determine whether the solution will of itself cause the formation of a precipitate of brownish-red cuprous oxide. If such a precipitate forms, the Fehling's solution must not be used. Add sugar solution to the warm Fehling's solution *a few drops* at a time and heat the mixture after each addition. The production of yellow cuprous hydroxide or brownish-red cuprous oxide indicates that reduction has taken place. The yellow precipitate is more likely to occur if the sugar solution is added rapidly and in large amount, whereas with a less rapid addition of smaller amounts of sugar solution the brownish-red precipitate is generally formed.

This is a much more satisfactory test than Trommer's, but even this test is not entirely reliable when used to detect sugar in the urine. Such bodies as *conjugate glycuronates*, *uric acid*, *nucleo-proteid* and *homogentisic acid* when present in sufficient amount may produce a result similar to that produced by sugar. Phosphates of the alkaline earths may be precipitated by the alkali of the Fehling's solution and in appearance may be mistaken for cuprous hydroxide. Cupric hydroxide may also be reduced to cuprous oxide and this in turn be dissolved by *creatinin*, a normal urinary constituent. This will give the urine under examination a greenish tinge and may obscure the sugar reaction even when a considerable amount of sugar is present.

(c) *Boettger's Test*.—To 5 c.c. of sugar solution in a test-tube add 1 c.c. of KOH or NaOH and a very small amount of bismuth subnitrate, and boil. The solution will gradually darken and finally assume a black color due to reduced bismuth. If the test is made on urine containing albumin this must be removed, by boiling and filtering, before applying the test, since with albumin a similar change of color is produced (bismuth sulphide).

(d) *Nylander's Test (Almén's Test)*.—To 5 c.c. of sugar solution in a test-tube add one-tenth its volume of Nylander's

reagent¹ and boil two or three minutes. The solution will darken if reducing sugar is present and upon standing for a few moments a black color will appear. This color is due to the precipitation of bismuth. If the test is made on urine containing albumin this must be removed, by boiling and filtering, before applying the test. It is claimed by Bechold that Nylander's and Boettger's tests give a negative reaction with solutions containing sugar when mercuric chloride or chloroform is present, a claim which Zeidlitz has very recently shown to be incorrect.

A positive Nylander or Boettger test is probably due to the following reactions:

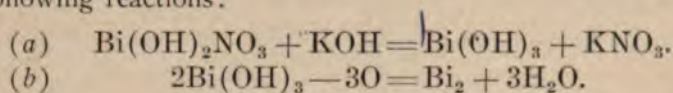


FIG. 2.



EINHORN SACCHAROMETER.

10. **Fermentation Test.** — "Rub up" in a mortar about 20 c.c. of the sugar solution with a small piece of compressed yeast. Transfer the mixture to a saccharometer (shown in Fig. 2) and stand it aside in a warm place for about twelve hours. If the sugar is fermentable, alcoholic fermentation will occur and carbon dioxide will collect as a gas in the upper portion of the tube. On the completion of fermentation introduce a little potassium hydroxide solution into the graduated portion by means of a bent pipette, place the thumb *tightly* over the opening in the apparatus and invert the saccharometer. Explain the result.

¹ Nylander's reagent is prepared by digesting 2 grams of bismuth subnitrate and 4 grams of Rochelle salt in 100 c.c. of a 10 per cent potassium hydroxide solution. The reagent is then cooled and filtered.

11. **Barfoed's Test.**—To 2–3 c.c. of Barfoed's solution¹ in a test-tube add a few drops of dextrose solution, and boil. Allow to stand a few moments and examine. Observe the red precipitate. What is it?

12. **Formation of Caramel.**—Gently heat a small amount of pulverized dextrose in a test-tube. After the sugar has melted and turned brown, allow the tube to cool, add water and warm. The coloring matter produced is known as *caramel*.

✓ 13. **Demonstration of Optical Activity.**—A demonstration of the use of the polariscope, by the instructor, each student being required to take readings and compute the "specific rotation."

USE OF THE POLARISCOPE.

For a detailed description of the different forms of polariscopes, the method of manipulation and the principles involved the student is referred to any standard text-book of physics. A brief description follows: An ordinary ray of light vibrates in every direction. If such a ray is caused to pass through a "polarizing" Nicol prism it is resolved into *two rays*, one of which vibrates in every direction as before and a second ray which vibrates in *one plane only*. This latter ray is said to be *polarized*. Many organic substances (sugars, proteids, etc.) have the power of twisting or rotating this plane of polarized light, the extent to which the plane is rotated depending upon the number of molecules which the polarized light passes. Substances which possess this power are said to be "optically active." The *specific rotation* of a substance is the rotation expressed in degrees which is afforded by one gram of substance dissolved in 1 c.c. of water in a tube one decimeter in length. The specific rotation, $(\alpha)_D$, may be calculated by means of the following formula,

¹ Barfoed's solution is prepared as follows: Dissolve 4 grams of copper acetate in 100 c.c. of water and acidify with acetic acid.

$$(\alpha)_D = \frac{\alpha}{p \cdot l},$$

in which

D = sodium light.

α = observed rotation in degrees.

p = grams of substance dissolved in 1 c.c. of liquid.

l = length of the tube in decimeters.

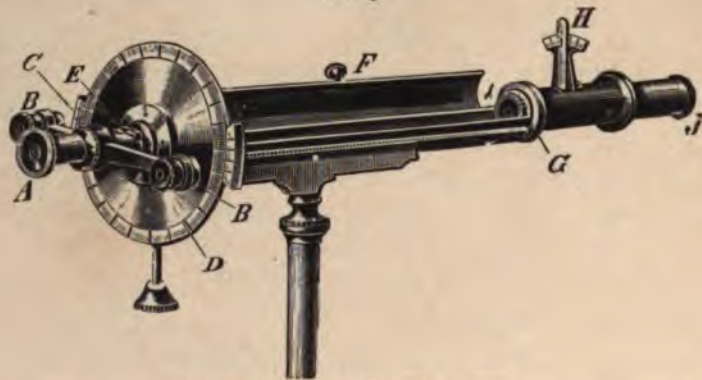
If the specific rotation has been determined and it is desired to ascertain the per cent of the substance in solution, this may be obtained by the use of the following formula,

$$p = \frac{\alpha}{(\alpha)_D \cdot l}.$$

The value of p multiplied by 100 will be the percentage of the substance in solution.

An instrument by means of which the extent of the rotation may be determined is called a *polariscope* or *polarimeter*. Such an instrument designed especially for the examination of sugar solutions is termed a *saccharimeter* or *polarizing saccharimeter*. The form of polariscope shown in Fig. 3, below,

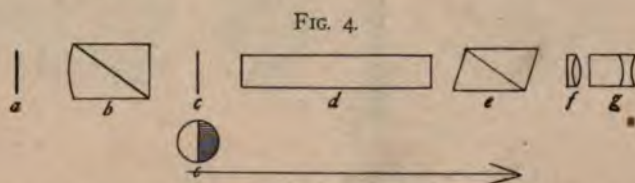
FIG. 3.



ONE FORM OF LAURENT POLARISCOPE.

B , Microscope for reading the scale; C , a vernier; E , position of the analyzing Nicol prism; H , polarizing Nicol prism in the tube below this point.

consists essentially of a long barrel provided with a Nicol prism at either end (Fig. 4, below). The solution under examination is contained in a tube which is placed between these two prisms. At the front end of the instrument is an adjusting eye-piece for focusing and a large recording disc which registers in degrees and fractions of a degree. The light is admitted into the far end of the instrument and is polarized by passing through a Nicol prism. This polarized ray then traverses the column of liquid within the tube mentioned above and if the substance is optically active the plane of the polarized ray is rotated to the right or left. Bodies rotating the ray to the right are called *dextro-rotatory* and those rotating it to the left *levo-rotatory*.



DIAGRAMMATIC REPRESENTATION OF THE COURSE OF THE LIGHT THROUGH THE LAURENT POLARISCOPE. (The direction is reversed from that of Fig. 3, p. 12.)

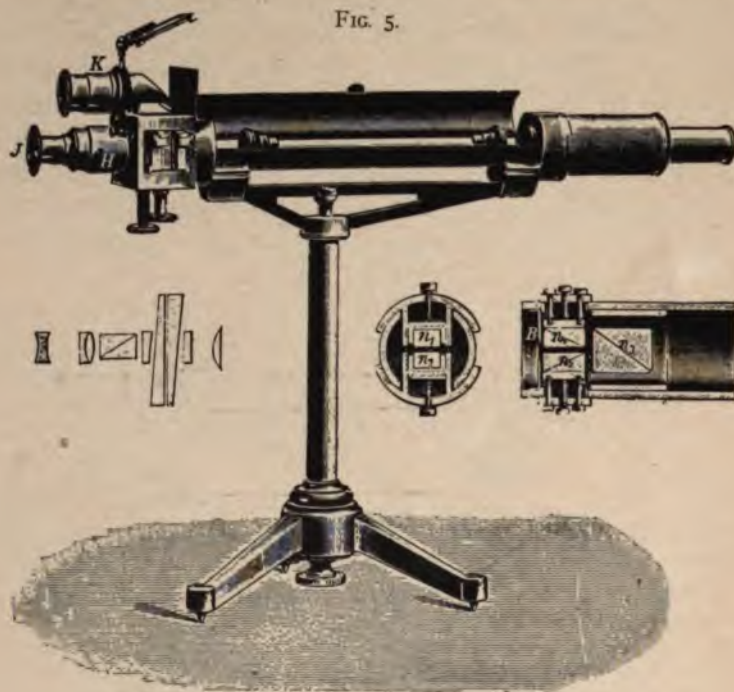
a, Bichromate plate to purify the light; *b*, the polarizing Nicol prism; *c*, a thin quartz plate covering one-half the field and essential in producing a second polarized plane; *d*, tube to contain the liquid under examination; *e*, the analyzing Nicol prism; *f* and *g*, ocular lenses.

Within the apparatus is a disc which is so arranged as to be without lines and uniformly light at *zero*. Upon placing the optically active substance in position, however, the plane of polarized light is rotated or turned and it is necessary to rotate the disc through a certain number of degrees in order to secure the normal conditions, *i. e.*, "without lines and uniformly light." The difference between this reading and the *zero* is α or the observed rotation in degrees.

Polarizing saccharimeters are also constructed by which the percentage of sugar in solution is determined by making an observation and multiplying the value of each division on a

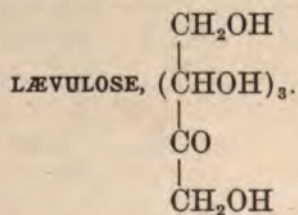
horizontal sliding scale by the value of the division expressed in terms of dextrose. The value, in terms of dextrose, of each of the divisions on the scale of the Laurent saccharimeter used in the laboratory of physiological chemistry at the University

FIG. 5.



POLARISCOPE (SCHMIDT AND HÄNSCH MODEL).

of Pennsylvania is 0.2051. This factor may vary according to the instrument.



As already stated, lævulose, sometimes called fructose or fruit sugar, occurs widely disseminated throughout the plant

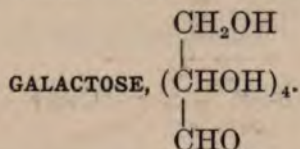
kingdom in company with dextrose. Its reducing power is somewhat weaker than that of dextrose. Lævulose does not ordinarily occur in the urine in diabetes mellitus, but has been found in exceptional cases. With phenylhydrazin it forms the same osazon as dextrose. With methylphenylhydrazin, lævulose forms a characteristic lævulose-methylphenylosazon.

(For a further discussion of lævulose see the section on Hexoses, p. 3.)

EXPERIMENTS ON LÆVULOSE.

1. **Seliwanoff's Reaction.**—If a solution of resorcin in dilute HCl (1 vol. concentrated HCl to 2 vols. H₂O), be warmed with lævulose the liquid will become red and a precipitate will separate. The precipitate may be dissolved in alcohol to which it will impart a striking red color. ✓

2. **Phenylhydrazin Test.**—Make the test according to directions under Dextrose, 3 or 4, pages 5 and 6.



Galactose occurs with dextrose as one of the products of the hydrolysis of lactose. It is dextro-rotatory, forms an osazon with phenylhydrazin and ferments slowly with yeast.

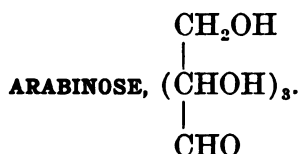
EXPERIMENTS ON GALACTOSE.

1. **Tollens' Reaction.**—To 5 c.c. of hydrochloric acid, having a specific gravity of 1.09, add a slight excess of phloroglucin, the acid being kept on a boiling water-bath during the addition. A few cubic centimeters of galactose solution should now be added and the heating continued. A red color is produced. Compare this color with that given by pentoses (see page 16).

2. **Phenylhydrazin Test.**—Make the test according to directions given under Dextrose, 3 or 4, pages 5 and 6. ✓

Pentoses, $C_5H_{10}O_5$.

In plants and more particularly in certain gums, very complex carbohydrates, called pentosans, occur. These pentosans through hydrolysis by acids may be transformed into pentoses. Pentoses do not ordinarily occur in the animal organism, but have been found in the urine of morphine habitués and others, their occurrence sometimes being a persistent condition without known cause. They are non-fermentable, have strong reducing power, and form osazons with phenylhydrazin. Pentoses are an important constituent of the dietary of herbivorous animals. Glycogen is said to be formed after the ingestion of these sugars containing five carbon atoms. On distillation with strong hydrochloric acid pentoses and pentosans yield furfural, which can be detected by its characteristic red reaction with aniline-acetate paper.



Arabinose, one of the most important pentoses, may be obtained from gum arabic, plum or cherry gum by boiling for several hours with 1-2 per cent sulphuric acid. It is dextro-rotatory, forms an osazon and has reducing power.

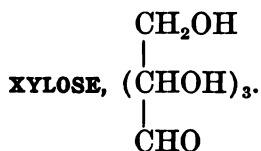
EXPERIMENTS ON ARABINOSE.

1. Tollens' Reaction.—To equal volumes of arabinose solution and hydrochloric acid (sp. gr. 1.09) add a little phloroglucin and heat the mixture on a boiling water-bath. Galactose, lævulose, pentose or glycuronic acid will be indicated by the appearance of a red color. To differentiate between these bodies make a spectroscopic examination and look for the absorption band between *D* and *E* given by pentoses and glycuronic acid. Differentiate between the two latter bodies by the melting-points of their osazons.

Compare the reaction with that obtained with galactose (page 15).

2. **Orcin Test.**—Repeat 1, using orcin instead of phloroglucin. A succession of colors from red through reddish-blue to green is produced. A green precipitate is formed which is soluble in amyl alcohol and has absorption bands between *C* and *D*.

3. **Phenylhydrazin Test.**—Make this test on the arabinose solution according to directions given under Dextrose, 3 or 4, pages 5 and 6.



Xylose, or wood sugar, is obtained by boiling wood gums with dilute acids as explained under Arabinose, page 16. It is dextro-rotatory and forms an osazon.

EXPERIMENTS ON XYLOSE.

1-3. Same as for arabinose (see page 16).

RHAMNOSE, $\text{C}_6\text{H}_{12}\text{O}_5$.

Rhamnose or methyl-pentose is an example of a true carbohydrate which does not have the H and O atoms present in the proportion to form water. Its formula is $\text{C}_6\text{H}_{12}\text{O}_5$. It has been found that rhamnose when ingested by rabbits or hens has a positive influence upon the formation of glycogen in those organisms.

DISACCHARIDES, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$.

The disaccharides as a class may be divided into two rather distinct groups. The first group would include those disaccharides which are found in nature as such, *e. g.*, *saccharose* and *lactose*, and the second group would include those disac-

charides formed in the hydrolysis of more complex carbohydrates, *e. g.*, *maltose* and *iso-maltose*.

The disaccharides have the general formula $C_{12}H_{22}O_{11}$, to which, in the process of hydrolysis, a molecule of water is added causing the single disaccharide molecule to split into two monosaccharide (hexose) molecules.

All of the more common disaccharides *except saccharose* have the power of reducing certain metallic oxides in alkaline solution, notably those of copper and bismuth. This reducing power is due to the presence of the *aldehyde group* ($-CHO$) in the sugar molecule.

Reduction ✓

MALTOSE, $C_{12}H_{22}O_{11}$.

Maltose or malt sugar is formed in the hydrolysis of starch through the action of a ferment, *diastase*, contained in sprouting barley or malt. Certain enzymes in the saliva and in the pancreatic juice may also cause a similar hydrolysis. Maltose is also an intermediate product of the action of dilute mineral acids upon starch. It is strongly dextro-rotatory, reduces metallic oxides in alkaline solution and is fermentable by yeast after being inverted (see Polysaccharides, page 21) by the enzyme *maltase* of the yeast. In common with the other disaccharides, maltose may be hydrolyzed with the formation of two molecules of monosaccharide. In this instance the products are two molecules of dextrose. With phenylhydrazin maltose forms an osazon, *maltosazon*.

EXPERIMENTS ON MALTOSE.

I-II. Repeat these experiments as given under Dextrose, pages 4-II.

ISO-MALTOSE, $C_{12}H_{22}O_{11}$.

Iso-maltose, an isomeric form of maltose, is formed, along with maltose, by the action of *diastase* upon starch paste, and also by the action of hydrochloric acid upon dextrose. It also occurs with maltose as one of the products of salivary digestion. It is dextro-rotatory and with phenylhydrazin gives an

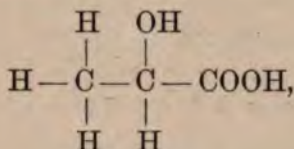
osazon which is characteristic. Iso-maltose is very soluble and reduces the oxides of bismuth and copper in alkaline solution. Pure iso-maltose is probably only slightly fermentable.

LACTOSE, $C_{12}H_{22}O_{11}$.

Reducing ✓

Lactose or milk sugar occurs ordinarily only in milk, but has often been found in the urine of women during pregnancy and lactation. It may also occur in the urine of normal persons after the ingestion of unusually large amounts of lactose in the food. It has a strong reducing power, is dextro-rotatory and forms an osazon with phenylhydrazin. Upon hydrolysis lactose yields one molecule of dextrose and one molecule of galactose. ✓

In the souring of milk the bacterium lactis and certain other micro-organisms bring about lactic acid fermentation by transforming the lactose of the milk into lactic acid,



and alcohol. This same reaction may occur in the alimentary canal as the result of the action of putrefactive bacteria. In the preparation of kephyr and koumyss the lactose of the milk undergoes alcoholic fermentation, through the action of ferments other than yeast, and at the same time lactic acid is produced.

Lactose is *not* fermentable by pure yeast.

EXPERIMENTS ON LACTOSE.

I-II. Repeat these experiments as given under Dextrose, pages 4-II.

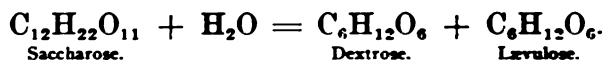
SACCHAROSE, $C_{12}H_{22}O_{11}$.

Saccharose, also called sucrose or cane sugar, is one of the most important of the sugars and occurs very extensively

3✓
4✓
5✓
6✓
7
8
9

distributed in plants, particularly in the sugar cane, sugar beet, sugar millet and in certain palms and maples.

Saccharose is dextro-rotatory and upon hydrolysis, as before mentioned, the molecule of saccharose takes on a molecule of water and breaks down into two molecules of monosaccharide. The monosaccharides formed in this instance are dextrose and lævulose. This is the reaction:



This process is called *inversion* and may be produced by weak acids, ferments and bacteria. After this inversion the previously strongly dextro-rotatory solution may be lævo-rotatory.

Saccharose does *not* reduce metallic oxides in alkaline solution and forms *no* osazon with phenylhydrazin. It is not fermentable directly by yeast, but must first be *inverted* by the ferment *invertin* contained in the yeast.

EXPERIMENTS ON SACCHAROSE.

1-11. Repeat these experiments according to the directions given under Dextrose, pages 4-11.

12. **Inversion of Saccharose.**—To 25 c.c. of saccharose solution in a beaker add 5 drops of concentrated HCl and boil one minute. Cool the solution, render alkaline with *solid* KOH and upon the resulting fluid repeat experiments 3 (or 4) and 9 as given under Dextrose, pages 5, 6 and 7. Explain the results.

13. **Production of Alcohol by Fermentation.**—Prepare a strong (10-20 per cent) solution of saccharose, add a small amount of egg albumin or commercial peptone and introduce the mixture into a bottle of appropriate size. Add yeast, and by means of a bent tube inserted through a stopper into the neck of the bottle, conduct the escaping gas into water. As fermentation proceeds readily in a warm place the escaping

gas may be collected in a eudiometer tube and examined. When the activity of the yeast has practically ceased, filter the contents of the bottle into a flask and distil the mixture. Catch the first portion of the distillate separately and test for alcohol by one of the following reactions:

(a) *Iodoform Test*.—Render 2–3 c.c. of the distillate alkaline with potassium hydroxide solution and add a few drops of iodine solution. Heat gently and note the formation of iodoform crystals. Examine these crystals under the microscope and compare them with those in Fig. 6.

(b) *Aldehyde Test*.—Place 5 c.c. of the distillate in a test-tube, add a few drops of potassium dichromate solution, $K_2Cr_2O_7$, and render it acid with dilute sulphuric acid. Boil the acid solution and note the odor of aldehyde.

FIG. 6.



IODOFORM. (Autenrieth.)

TRISACCHARIDES, $C_{18}H_{32}O_{16}$.

RAFFINOSE.

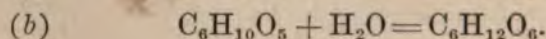
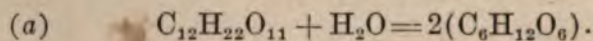
This trisaccharide, also called melitose or melitriose, occurs in cotton seed, Australian manna and in the molasses from the preparation of beet sugar. It is dextro-rotatory, does not reduce Fehling's solution and is only partially fermentable by yeast.

Raffinose may be hydrolyzed by weak acids the same as the polysaccharides are hydrolyzed, the products being dextrose and melibiose; further hydrolysis of the melibiose yields dextrose and galactose.

POLYSACCHARIDES, $(C_6H_{10}O_5)_x$.

In general the polysaccharides are amorphous bodies, a few, however, are crystallizable. Through the action of certain

enzymes or weak acids the polysaccharides may be hydrolyzed with the formation of monosaccharides. As a class the polysaccharides are quite insoluble and are non-fermentable until inverted. By inversion is meant the hydrolysis of disaccharide or polysaccharide sugars to form monosaccharides, as indicated in the following equations:



STARCH, $(C_6H_{10}O_5)_x$.

Starch is widely distributed throughout the vegetable kingdom, occurring in grains, fruits and tubers. It occurs in granular form, the microscopical appearance being typical for each individual starch. The granules, which differ in size according to the source, are composed of alternating concentric rings of granulose and cellulose. Ordinary starch is insoluble in cold water, but if boiled with water the cell walls are ruptured and *starch paste* results.

Starch is acted upon by diastatic enzymes, *e. g.*, *ptyalin* and *amyllopsin*, with the formation of *soluble starch*, *erythro-dextrin*, *achroo-dextrin*, *malto-dextrin*, *maltose*, *iso-maltose* and *dextrose* (see Salivary Digestion, page 34). Maltose is the principal end-product of this enzyme action. Upon boiling a starch solution with a dilute mineral acid a series of similar bodies is formed, but under these conditions *dextrose* is the principal end-product.

EXPERIMENTS ON STARCH.

1. **Preparation of Potato Starch.**—Pare a raw potato, comminute it upon a fine grater, mix with water, and “whip up” the pulped material vigorously before straining it through cheese cloth or gauze to remove the coarse particles. The starch rapidly settles to the bottom and can be washed by repeated decantation. Allow the compact mass of starch to drain thoroughly and spread it out on a watch glass to dry in

FIG. 7.



POTATO.

FIG. 8.



BEAN.

FIG. 9.



ARROWROOT.

FIG. 10.



RYE.

FIG. 11.



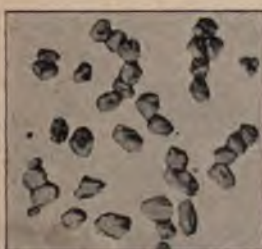
BARLEY.

FIG. 12.



OAT.

FIG. 13.



BUCKWHEAT.

FIG. 14.



MAIZE.

FIG. 15.



RICE.

FIG. 16.



PEA.

FIG. 17.



WHEAT.

STARCH GRANULES FROM VARIOUS SOURCES. (Leffmann and Beam.)

the air. If so desired this preparation may be used in the experiments which follow.

2. **Microscopical Examination.**—Examine microscopically the granules of the various starches submitted and compare them with those shown in Figs. 7-17, page 23.

✓ 3. **Solubility.**—Try the solubility of one form of starch in each of the ordinary solvents (see page 4). If uncertain regarding the solubility in any reagent, filter and test the filtrate with iodine solution as given under 5 below. The production of a blue color would indicate that the starch had been dissolved by the solvent.

✓ 4. **Iodine Test.**—Place a few granules of starch in one of the depressions of a porcelain test-tablet and treat with a drop of a dilute solution of iodine in potassium iodide. The granules are colored blue due to the formation of so-called *iodide of starch*. The cellulose of the granule is not stained as may be seen by examining microscopically.

✓ 5. **Iodine Test on Starch Paste.**—Repeat the iodine test using the starch paste. Place 2-3 c.c. of starch paste¹ in a test-tube, add a drop of the dilute iodine solution and observe the production of a blue color. Heat the tube and note the disappearance of the color. It reappears on cooling.

In similar tests note the influence of alcohol and of alkali upon the so-called iodide of starch.

The composition of the iodide of starch is not definitely known.

✓ 6. **Fehling's Test.**—On starch paste (see page 8).

✓ 7. **Hydrolysis of Starch.**—Place about 25 c.c. of starch paste in a small beaker, add 10 drops of concentrated HCl, and boil. By means of a small pipette, at the end of each minute, remove a drop of the solution to the test-tablet and make

¹ *Preparation of Starch Paste.*—Grind 2 grams of starch powder in a mortar with a small amount of cold water. Bring 200 c.c. of water to the boiling-point and add the starch mixture from the mortar with continuous stirring. Bring again to the boiling-point and allow it to cool. This makes an approximate 1 per cent starch paste which is a very satisfactory strength for general use.

the regular iodine test. As the testing proceeds the blue color should gradually fade and finally disappear. At this point, after cooling and neutralizing with solid KOH, Fehling's test (see p. 8) should give a positive result due to the formation of a reducing sugar from the starch. Make the phenylhydrazin test upon some of the hydrolyzed starch. Try also Barfoed's test (see p. 11). What sugar has been formed?

✓ 8. **Influence of Tannic Acid.**—Add an excess of tannic acid solution to a small amount of starch paste in a test-tube. The liquid will become strongly opaque and ordinarily a yellowish-white precipitate is produced. Compare this result with the result of the similar experiment on dextrin (p. 28).

9. **Diffusibility of Starch Paste.**—Test the diffusibility of starch paste through animal membrane or parchment paper, making a dialyzer like one of the models shown in Fig. 1, page 6.

INULIN, $(C_6H_{10}O_5)_x$

Inulin is a polysaccharide which may be obtained as a white, odorless, tasteless powder from the tubers of the artichoke, elecampane or dahlia. It has also been prepared from the roots of chicory, dandelion and burdock. It is very slightly soluble in cold water and quite easily soluble in hot water. In cold alcohol of 60 per cent or over it is practically insoluble. Inulin gives a negative reaction with iodine solution. The "yellow" color reaction with iodine mentioned in many books is doubtless merely the normal color of the iodine solution. It is very difficult to prepare inulin which does not reduce Fehling's solution slightly. This reducing power may be due to an impurity. Practically all commercial preparations of inulin possess considerable reducing power.

Inulin is lævo-rotatory and upon hydrolysis by acids or by the enzyme *inulase* it yields the monosaccharide lævulose which readily reduces Fehling's solution. The ordinary amylolytic enzymes occurring in the animal body do not digest inulin.

EXPERIMENTS ON INULIN.

1. **Solubility.**—Try the solubility of inulin powder in each of the ordinary solvents. If uncertain regarding the solubility in any reagent, filter and neutralize the filtrate if it is alkaline in reaction. Add a drop of concentrated hydrochloric acid to the filtrate and boil it for one minute. Render the solution neutral or slightly alkaline with *solid* KOH and try Fehling's test. What is the significance of a positive Fehling's test in this connection?

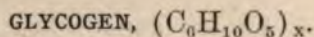
2. **Iodine Test.**—(a) Place 2–3 c.c. of the inulin solution in a test-tube and add a drop of dilute iodine solution. What do you observe?

(b) Place a small amount of inulin powder in one of the depressions of a test-tablet and add a drop of dilute iodine solution. Is the effect any different from that observed above?

3. **Molisch's Reaction.**—Repeat this test according to directions given under Dextrose, 2, page 4.

4. **Fehling's Test.**—Make this test on the inulin solution according to the instructions given under Dextrose, page 8. Is there any reduction?¹

5. **Hydrolysis of Inulin.**—Place 5 c.c. of inulin solution in a test-tube, add a drop of concentrated hydrochloric acid and boil it for one minute. Now cool the solution, neutralize it with concentrated KOH and test the reducing action of 1 c.c. of the solution upon 1 c.c. of diluted (1:4) Fehling's solution. Explain the result.²



(For discussion and experiments see Muscular Tissue, page 206.)

¹ See the discussion of the properties of inulin, page 25.

² If the inulin solution gave a positive Fehling test in the last experiment it will be necessary to check the hydrolysis experiment as follows: To 5 c.c. of the inulin solution in a test-tube add one drop of concentrated hydrochloric acid, neutralize with concentrated KOH solution and test the reducing action of 1 c.c. of the resulting solution upon 1 c.c. of diluted (1:4) Fehling's solution. This will show the normal reducing power of the inulin solution. In case the inulin was hydrolyzed, the Fehling's

LICHENIN, $(C_6H_{10}O_5)_x$.

Lichenin may be obtained from *Cetraria islandica* (Iceland moss). It forms a difficultly soluble jelly in cold water and an opalescent solution in hot water. It is optically inactive and gives no color with iodine. Upon hydrolysis with dilute mineral acids lichenin yields dextrins and dextrose. It is said to be most nearly related chemically to starch. Saliva, pancreatic juice, malt diastase and gastric juice have no noticeable action on lichenin.

DEXTRIN, $(C_6H_{10}O_5)_x$.

The dextrins are the bodies formed midway in the stages of the hydrolysis of starch by weak acids or an enzyme. They are amorphous bodies which are easily soluble in water, acids and alkalis but are insoluble in alcohol or ether. Dextrins are dextro-rotatory and are not fermentable by yeast.

The dextrins may be hydrolyzed by dilute acids to form dextrose. With iodine one form of dextrin (erythro-dextrin) gives a red color. Their power to reduce Fehling's solution is questioned.

EXPERIMENTS ON DEXTRIN.

1. **Solubility.**—Test the solubility of pulverized dextrin in the ordinary solvents (see page 4).

2. **Iodine Test.**—Place a drop of dextrin solution in one of the depressions of the test-tablet and add a drop of a dilute solution of iodine in potassium iodide. A red color results. If the reaction is not sufficiently pronounced make a stronger solution from the pulverized dextrin and repeat the test. The solution should be slightly acid to secure the best results.

3. **Fehling's Test.**—See if the dextrin solution will reduce Fehling's solution.

4. **Hydrolysis of Dextrin.**—Take 25 c.c. of dextrin solution in a small beaker, add 5 drops of dilute HCl, and boil.

test in the hydrolysis experiment should show a more pronounced reduction than that observed in the check experiment.

By means of a small pipette, at the end of each minute, remove a drop of the solution to one of the depressions of the test-tablet and make the iodine test. The power of the solution to produce a color with iodine should rapidly disappear. When a negative reaction is obtained cool the solution and neutralize it with *solid* KOH. Try Fehling's test (see page 8). This reaction is now strongly positive, due to the formation of a reducing sugar. Determine the nature of the sugar by means of the phenylhydrazin test (see pages 5 and 6).

5. **Influence of Tannic Acid.**—Add an excess of tannic acid solution to a small amount of dextrin solution in a test-tube. No precipitate forms. This result differs from the result of the similar experiment upon starch (see Starch, 8, page 25).

6. **Diffusibility of Dextrin.**—(See Starch, 9, page 25.)

7. **Precipitation by Alcohol.**—To about 50 c.c. of 95 per cent alcohol in a small beaker add about 10 c.c. of a *concentrated* dextrin solution. Dextrin is thrown out of solution as a gummy white precipitate. Compare the result with that obtained under Dextrose, 5, page 6.

CELLULOSE, $(C_6H_{10}O_5)_x$.

This complex polysaccharide forms a large portion of the cell wall of plants. It is extremely insoluble and its molecule is much more complex than the starch molecule. The best quality of filter paper and the ordinary absorbent cotton are good types of cellulose.

EXPERIMENTS ON CELLULOSE.

✓ 1. **Solubility.**—Test the solubility of cellulose in the ordinary solvents (see page 4).

✓ 2. **Iodine Test.**—Add a drop of dilute iodine solution to a few shreds of cotton on a test-tablet. Cellulose differs from starch and dextrin in giving *no color* with iodine.

✓ 3. **Formation of Amyloid.**¹—Add 10 c.c. of dilute and 5

¹ This body derives its name from *amylum* (starch) and is not to be confounded with amyloid, the gluco-proteid (page 62).

c.c. of concentrated H_2SO_4 to some absorbent cotton in a test-tube. When entirely dissolved (without heating) pour one-half of the solution into another test-tube, cool it and dilute with water. Amyloid forms as a gummy precipitate and gives a brown or blue coloration with iodine.

After allowing the second portion of the acid solution of cotton to stand about 10 minutes dilute it with water in a small beaker and boil for 15-30 minutes. Now cool, neutralize with *solid* KOH and test with Fehling's solution. Dextrose has been formed from the cellulose by the action of the acid.

4. **Schweitzer's Solubility Test.**—Heat some absorbent cotton in a test-tube with Schweitzer's reagent.¹ When completely dissolved acidify the solution with acetic acid. An amorphous precipitate of cellulose is produced. Schweitzer's reagent is the *only solvent* for cellulose.

REVIEW OF CARBOHYDRATES.

In order to facilitate the student's review of the carbohydrates, the preparation of a chart similar to the appended

MODEL CHART FOR REVIEW PURPOSES.

Carbohydrate.	Solubility.	Iodine Test	Moore's Test.	Trommer's Test.	Fehling's Test.	Boettger's Test.	Nylander's Test	Barfoed's Test.	Molisch's Reaction.	Precipitation by Alcohol.	Osazon.	Rotation.	D fusibility	Fermentation.	Remarks.
Dextrose.															
Maltose.															
Lactose.															
Saccharose.															
Starch.															
Inulin.															
Dextrin.															
Cellulose.															

¹ Schweitzer's reagent is made by adding potassium hydroxide to a solution of cupric sulphate which contains some ammonium chloride. A precipitate of cupric hydroxide forms and this is filtered off, washed and brought into solution in 20 per cent ammonium hydroxide.

model is recommended. The signs + and — may be conveniently used to indicate positive and negative reaction. Only those carbohydrates which are of greatest importance from the standpoint of physiological chemistry have been included in the chart.

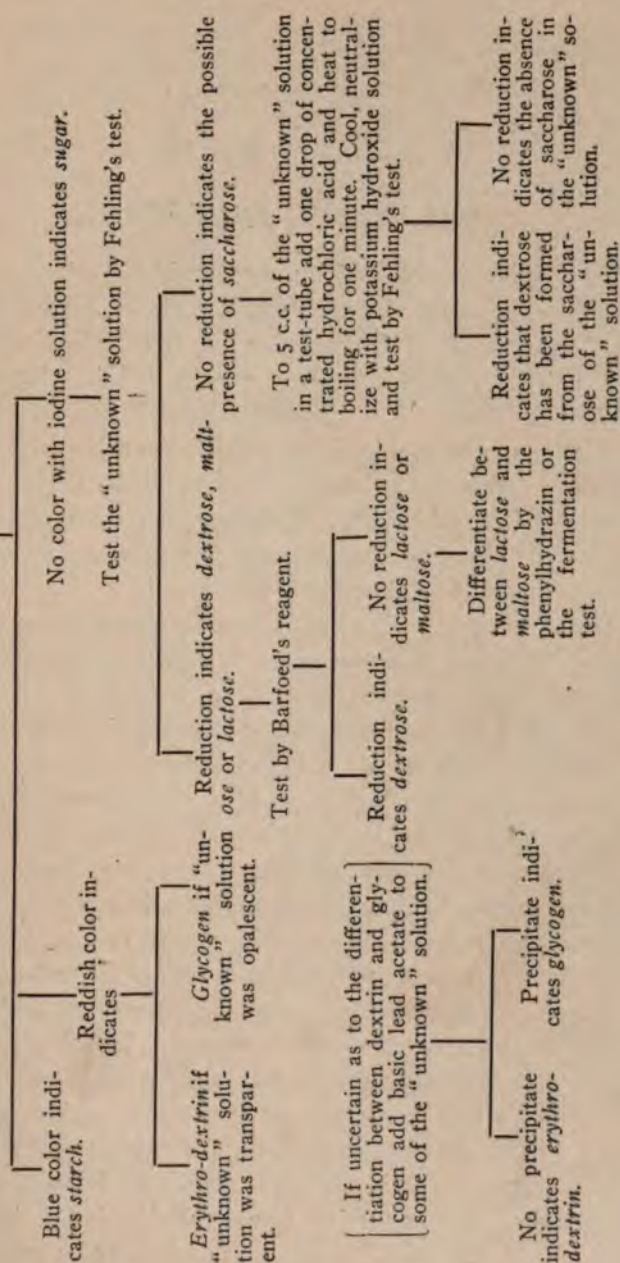
“ UNKNOWN ” SOLUTIONS OF CARBOHYDRATES.

At this point the student will be given several “ unknown ” solutions, each solution containing one or more of the carbohydrates studied. He will be required to detect, by means of the tests on the preceding pages, each carbohydrate constituent of the several “ unknown ” solutions and hand in, to the instructor, a written report of his findings, on slips furnished by the laboratory.

The scheme given on page 31 may be of use in this connection.

SCHEME FOR THE DETECTION OF CARBOHYDRATES.

If the solution is alkaline, neutralize or make faintly acid with HCl. Test a few drops on a test-tablet with iodine solution.



CHAPTER II.

SALIVARY DIGESTION.

THE saliva is secreted by three pairs of glands, the submaxillary, sublingual and parotid, reinforced by numerous small glands called buccal glands. The saliva secreted by each pair of glands possesses certain definite characteristics peculiar to itself. For instance, in man, the parotid glands ordinarily secrete a thin, watery fluid, the submaxillary glands secrete a somewhat thicker fluid containing mucin, while the product of the sublingual glands has a more mucilaginous character. The saliva as collected from the mouth is the combined product of all the glands mentioned.

The saliva may be induced to flow by many forms of stimuli, such as *chemical, mechanical, electrical, thermal* and *psychical*, the nature and amount of the secretion depending, to a limited degree, upon the particular class of stimuli employed as well as upon the character of the individual stimulus. For example, in experiments upon dogs it has been found that the mechanical stimulus afforded by dropping several pebbles into the animal's mouth caused the flow of but one or two drops of saliva, whereas the mechanical stimulus afforded by sand in the mouth induced a copious flow of a thin watery fluid. Again, when ice-water or snow was placed in the animal's mouth no saliva was seen, while an acid or anything possessing a bitter taste, which the dog wished to reject, caused a free flow of the thin saliva. On the other hand, when articles of food were placed in the dog's mouth the animal secreted a thicker saliva having a higher mucin content—a fluid which would lubricate the food and assist in the passage of the bolus through the œsophagus. It was further found that by simply drawing the attention of the animal to any of the substances named above.

results were obtained similar to those secured when the substances were actually placed in the animal's mouth. For example, when a pretense was made of throwing sand into the dog's mouth, a watery saliva was secreted, whereas food under the same conditions excited a thicker and more slimy secretion. The exhibition of dry food, in which the dog had no particular interest (dry bread) caused the secretion of a large amount of saliva, while the presentation of moist food, which was eagerly desired by the animal, called forth a much smaller secretion. These experiments show it to be rather difficult to differentiate between the influence of physiological and psychical stimuli.

The amount of saliva secreted by an adult in 24 hours has been variously placed, as the result of experiment and observation, between 1000 and 1500 c.c., the exact amount depending, among other conditions, upon the character of the food.

The saliva ordinarily has a weak, alkaline reaction to litmus, but becomes acid 2-3 hours after a meal or during fasting. The alkalinity is due principally to di-sodium hydrogen phosphate (Na_2HPO_4) and its average alkalinity may be said to be equivalent to 0.08 — 0.1 per cent sodium carbonate. The saliva is the most dilute of all the digestive fluids, having an average specific gravity of 1.005 and containing only 0.5 per cent of solid matter. Among the solids are found albumin, globulin, mucin, urea, the enzyme ptyalin, phosphates and other inorganic constituents. Potassium sulphocyanide, KSCN, is also generally present in the saliva. It has been claimed that this substance is present in greatest amount in the saliva of habitual smokers. The significance of sulphocyanide in the saliva is not known; it may come from the breaking down of proteid.

The so-called tartar formation on the teeth is composed almost entirely of calcium phosphate with some calcium carbonate, mucin, epithelial cells and organic debris derived from the food. The calcium salts are held in solution as acid salts,

and are probably precipitated by the ammonia of the breath. The various organic substances just mentioned are carried down in the precipitation of the calcium salts.

The saliva contains an *enzyme* known as *ptyalin*. This is an *amylolytic* enzyme, so-called because it possesses the property of transforming complex carbohydrates such as starch and dextrin into simpler bodies. The so-called *ferments* were formerly divided into two general groups, (1) *true ferments* or so-called organized ferments such as yeast and certain bacteria, which were supposed to act by virtue of vital processes; and (2) *enzymes* such as ptyalin, which are non-living, unorganized bodies of a chemical nature. Recently this distinction between true ferments and enzymes has been proven to be incorrect since it has been shown that certain of the bodies formerly supposed to derive their ferment activity by virtue of their vital processes in reality secrete certain definite enzymes which are solely responsible for their ferment activity. In no sense is it a vital process since the ferment activity is entirely independent of the vital processes of the cell. We may define an enzyme as *an unorganized, soluble ferment which is elaborated by an animal or vegetable cell and whose activity is entirely independent of any of the life processes of such a cell.*

The more important enzymes may be classified, according to the character of their action, as follows: (1) *amylolytic* (starch transforming), (2) *proteolytic* (proteid transforming), (3) *adipolytic* or *lipolytic* (fat splitting), (4) *inverting* (possesses inverting power), (5) *oxidative* (possesses oxidizing power), and (6) *proteid coagulating*.

The action of ptyalin is one of hydrolysis and through this action a series of simpler bodies are formed from the complex starch. The first product of the action of the ptyalin of the saliva upon starch paste is *soluble starch* (amidulin) and its formation is indicated by the disappearance of the opalescence of the starch solution. This body resembles true starch in giving a blue color with iodine. Next follows the formation, in succession, of a series of dextrans, called *erythro-dextrin*,

achroo-dextrin and *malto-dextrin*, the *erythro-dextrin* being formed directly from the *soluble starch* and later being itself transformed into *achroo-dextrin* from which in turn is produced *malto-dextrin*. Accompanying each dextrin a small amount of maltose is formed, the quantity of maltose growing gradually larger as the process of transformation progresses. Erythro-dextrin gives a red color with iodine, the other dextrans give no color. The next stage is the transformation of the *malto-dextrin* into *maltose* the latter being the principal end-product of the salivary digestion of starch. At this point small amounts of *iso-maltose* and *dextrose* are formed from the maltose, the dextrose being produced through the action of the enzyme *maltase*.

Ptyalin acts in alkaline or neutral solutions. It will also act in the presence of relatively strong *combined* HCl (see page 84), whereas a trace (0.003 per cent to 0.006 per cent) of ordinary *free* hydrochloric acid will not only prevent the action but will destroy the enzyme. By sufficiently increasing the alkalinity of the saliva the action of the ptyalin is inhibited. It has recently been shown, by Cannon, to be strongly probable that salivary digestion may proceed for a considerable period after the food reaches the stomach, owing to the slowness with which the contents are thoroughly mixed with the acid gastric juice and the consequent tardy destruction of the enzyme.

Microscopical examination of the saliva reveals salivary corpuscles, bacteria, food debris, epithelial cells, mucus and fungi. In certain pathological conditions of the mouth, pus cells and blood corpuscles may be found in the saliva.

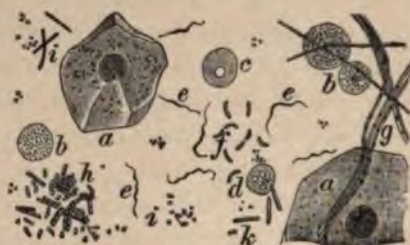
EXPERIMENTS ON SALIVA.

A satisfactory method of obtaining the saliva necessary for the experiments which follow is to chew a small piece of pure paraffin wax thus stimulating the flow of the secretion, which may be collected in a small beaker. Filtered saliva is to be used in every experiment except for the microscopical examination.

1. **Microscopical Examination.**—Examine a drop of unfiltered saliva microscopically and compare with Fig. 18 below.

2. **Reaction.**—Test the reaction to litmus.

FIG. 18.



MICROSCOPICAL CONSTITUENTS OF SALIVA.

a, Epithelial cells; *b*, salivary corpuscles; *c*, fat drops; *d*, leucocytes; *e*, *f* and *g*, bacteria; *h*, *i* and *k*, fission-fungi.

3. **Specific Gravity.**—Partially fill a urinometer cylinder with saliva, introduce the urinometer (see Fig. 83, page 232), and observe the reading.

4. **Test for Mucin.**—To a small amount of saliva in a test-tube add 1–2 drops of dilute acetic acid. Mucin is precipitated.

5. **Biuret Test.**¹—Render a little saliva alkaline with an equal volume of KOH and add a few drops of a *very dilute* (2–5 drops in a test-tube of water) cupric sulphate solution. The formation of a purplish-violet color is due to mucin.

6. **Millon's Reaction.**²—Add a few drops of Millon's reagent to a little saliva. A light yellow precipitate formed by the mucin gradually turns red upon being *gently* heated.

7. **Preparation of Mucin.**—Pour 15 c.c. of saliva into 100 c.c. of 95 per cent alcohol, stirring constantly. Cover the vessel and allow the precipitate to stand at least 12 hours. Pour off the supernatant liquid, collect the precipitate on a filter and wash it, in turn, with alcohol and ether. Finally dry the precipitate, remove it from the paper and make the following tests on the mucin: (*a*) Test its solubility in the ordinary

¹ The significance of this reaction is pointed out on page 45.

² The significance of this reaction is pointed out on page 44.

solvents (see page 4), (b) Millon's reaction, (c) dissolve a small amount in KOH, and try the biuret test on the solution, (d) boil the remainder, with 10–25 c.c. of water to which 5 c.c. of dilute HCl has been added, until the solution becomes brownish. Cool, render alkaline with *solid* KOH, and test by Fehling's solution. A reduction should take place. Mucin is what is known as a compound proteid or glucoproteid (see p. 61) and upon boiling with the acid the carbohydrate group in the molecule has been split off from the proteid portion and its presence is indicated by the reduction of Fehling's solution.

8. Inorganic Matter.—Test for chlorides, phosphates, sulphates and calcium. For chlorides, acidify with HNO_3 and add AgNO_3 . For phosphates, acidify with HNO_3 , heat and add molybdic solution.¹ For sulphates, acidify with HCl and add BaCl_2 and warm. For calcium, acidify with acetic acid, CH_3COOH , and add ammonium oxalate, $(\text{NH}_4)_2\text{C}_2\text{O}_4$.

9. Filtration Experiment.—Place filter papers in two funnels, and to each add an equal quantity of starch paste (5 c.c.). Add a few drops of saliva to one lot of paste and an equivalent amount of water to the other. Note the progress of filtration in each case. Why does one solution filter more rapidly than the other?

10. Test for Nitrites.—Add 1–2 drops of dilute H_2SO_4 to a little saliva and thoroughly stir. Now add a few drops of a potassium iodide solution and some starch paste. Nitrous acid is formed which liberates iodine causing the formation of the blue iodide of starch.

11. Sulphocyanide Tests.—(a) *Ferric Chloride Test.*—To a little saliva in a small porcelain crucible, or dish, add a few drops of dilute ferric chloride and acidify slightly with HCl. Red ferric sulphocyanide forms. To show that the red coloration is not due to iron phosphate add a drop of HgCl_2 when *colorless* mercuric sulphocyanide forms.

¹ Molybdic solution is prepared as follows, the parts being by weight:

1 part, molybdic acid.

4 parts, ammonium hydroxide (Sp. gr. 0.96).

15 parts, nitric acid (Sp. gr. 1.2).

(b) *Solera's Reaction*.—This test depends upon the liberation of iodine through the action of sulphocyanide upon iodic acid. Moisten a strip of *starch paste-iodic acid* test paper¹ with a little saliva. If sulphocyanide be present the test paper will assume a blue color, due to the liberation of iodine and its subsequent formation of the so-called iodide of starch.

12. **Digestion of Starch Paste**.—To 25 c.c. of starch paste in a small beaker, add 5 drops of saliva and stir thoroughly. At intervals of a minute remove a drop of the solution to one of the depressions in a test-tablet and test by the iodine test. If the blue color with iodine still forms after 5 minutes, add another 5 drops of saliva. The opalescence of the starch solution should soon disappear, indicating the formation of *soluble starch* which gives a blue color with iodine. This body should soon be transformed into *erythro-dextrin* which gives a red color with iodine and this in turn should pass into *achroo-dextrin* which gives no color with iodine. This is called the *achromic point*. When this point is reached test by Fehling's test to show the production of a reducing body. A body formed coincidentally with erythro-dextrin may yield a slight response to Fehling's test. What body is it? How long did it take for a complete transformation of the starch?

13. **Digestion of Dry Starch**.—In a test-tube shake up a small amount of *dry starch* with a little water. Add a few drops of saliva, mix well and allow to stand. After 10–20 minutes filter and test the filtrate by Fehling's test. What is the result and why?

14. **Digestion of Inulin**.—To 5 c.c. of inulin solution in a test-tube add 10 drops of saliva and place the tube in the water-bath at 40° C. After one-half hour test the solution by Fehling's test.² Is any reducing substance present? What do you conclude regarding the salivary digestion of inulin?

¹ This test paper is prepared as follows: Saturate a good quality of filter paper with 0.5 per cent starch paste containing a little iodic acid and allow the paper to dry in the air. Cut it in strips of suitable size and preserve for use.

² If the inulin solution gives a reduction before being acted upon by the saliva it will be necessary to determine the extent of this original reduction by means of a "check" test (see page 26).

15. **Influence of Temperature.**—In each of four tubes place about 5 c.c. of starch paste. Immerse one tube in cold water from the faucet, keep a second at room temperature and place a third on the water-bath at 40°C . Now add to the contents of each of these three tubes two drops of saliva and shake well; to the contents of the fourth tube add two drops of *boiled* saliva. Test frequently by the iodine test, using the test-tablet, and note in which tube the most rapid digestion occurs. Explain the results.

16. **Influence of Dilution.**—Take a series of 6 test-tubes each containing 9 c.c. of water. Add 1 c.c. of saliva to tube 1 and shake thoroughly. Remove 1 c.c. of the solution from tube 1 to tube 2 and after mixing thoroughly remove 1 c.c. from tube 2 to tube 3. Continue in this manner until you have 6 saliva solutions of gradually decreasing strength. Now add starch paste in equal amounts to each tube, mix very thoroughly and place on the water-bath at 40°C . After 10–20 minutes test by both the iodine and Fehling's tests. In how great dilution does your saliva act?

17. **Influence of Acids and Alkalis.**—(a) *Influence of Free Acid.*—Prepare a series of 6 tubes in each of which is placed 4 c.c. of one of the following strengths of *free* HCl: 0.2 per cent, 0.1 per cent, 0.05 per cent, 0.025 per cent, 0.0125 per cent and 0.006 per cent. Now add 2 c.c. of starch paste to each tube and shake them thoroughly. Complete the solutions by adding 2 c.c. of saliva to each and repeat the shaking. The *total acidity* of this series would be as follows: 0.1 per cent, 0.05 per cent, 0.025 per cent, 0.0125 per cent, 0.006 per cent and 0.003 per cent. Place these tubes on the water-bath at 40°C . for 10–20 minutes. Divide the contents of each tube into two parts, testing one part by the iodine test and testing the other, after neutralization, by Fehling's test. What do you find?

(b) *Influence of Combined Acid.*—Repeat the first three experiments of the above series using *combined* hydrochloric acid (see page 84) instead of the *free* acid. How does the action of the *combined acid* differ from that of the *free acid*?

(c) *Influence of Alkali*.—Repeat the first four experiments under (a) replacing the HCl by 2 per cent, 1 per cent, 0.5 per cent and 0.25 per cent Na_2CO_3 . Neutralize the alkalinity before trying the iodine test (see Starch, 5, page 24).

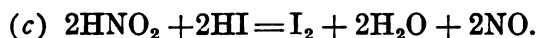
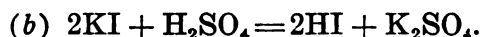
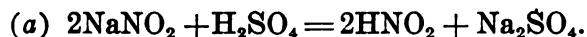
(d) *Nature of the Action of Acid and Alkali*.—Place 2 c.c. of saliva and 2 c.c. of 0.2 per cent HCl in a test-tube and leave for 15 minutes. Neutralize the solution, add 4 c.c. of starch paste and place the tube on the water-bath at 40°C . In 10 minutes test by the iodine and Fehling's tests and explain the result. Repeat the experiment replacing the 0.2 per cent HCl by 2 per cent Na_2CO_3 . What do you deduce from these two experiments?

18. **Influence of Metallic Salts, etc.**—In each of a series of tubes place 4 c.c. of starch paste and $\frac{1}{2}$ c.c. of one of the solutions named below. Shake well, add $\frac{1}{2}$ c.c. of saliva to each tube, thoroughly mix, and place on the water-bath at 40°C . for 10–20 minutes. Show the progress of digestion by means of the iodine and Fehling tests. Use the following chemicals: *Metallic salts*, 10 per cent plumbic acetate, 2 per cent cupric sulphate, 5 per cent ferric chloride, 8 per cent mercuric chloride; *Neutral salts*, 10 per cent sodium chloride, 3 per cent barium chloride, 10 per cent Rochelle salt. Also try the influence of 2 per cent carbolic acid, 95 per cent alcohol, and ether and chloroform. What are your conclusions?

19. **Excretion of Potassium Iodide**.—Ingest a small dose of potassium iodide (0.2 gram) contained in a gelatin capsule, quickly rinse out the mouth with water and then test the saliva at once for iodine. This test should be negative. Make additional tests for iodine at 2 minute intervals. The test for iodine is made as follows: Take 1 c.c. of NaNO_2 and 1 c.c. of dilute H_2SO_4 ¹ in a test-tube, add a little saliva directly from the mouth, and a small amount of starch paste. If convenient, the urine may also

¹ Instead of this mixture a few drops of HNO_3 possessing a yellowish or brownish color due to the presence of HNO_2 may be employed.

be tested. The formation of a blue color signifies that the potassium iodide is being excreted through the salivary glands. Note the length of time elapsing between the ingestion of the potassium iodide and the appearance of the first traces of the substance in the saliva. The chemical reactions taking place in this experiment are indicated in the following equations:



20. Qualitative Analysis of the Products of Salivary Digestion.—To 25 c.c. of the products of salivary digestion (saved from Experiment 12 or furnished by the instructor), add 100 c.c. of 95 per cent alcohol. Allow to stand until the white precipitate has settled. Filter, evaporate the filtrate to dryness, dissolve the residue in 5–10 c.c. of water and try Fehling's test (page 8) and the phenylhydrazin reaction (see Dextrose, 3, page 5). On the dextrin precipitate try the iodine test (page 24). Also hydrolyze the dextrin as given under Dextrin, 4, page 27.

CHAPTER III.

PROTEIDS.

Proteids are a group of very complex organic substances, constituting the most important class of food stuffs and are widely distributed in animal and vegetable tissues. Every proteid contains *carbon, hydrogen, nitrogen, oxygen and sulphur*, and a relatively small number contain *phosphorus and iron* in addition. The percentage composition of the more important members of the group would fall within the following limits: C (51 per cent to 55 per cent), H (6 per cent to 7.3 per cent), N (15 per cent to 19 per cent), O (21 per cent to 23 per cent), S (0.3 per cent to 2.5 per cent), and P (0.4 per cent to 0.8 per cent *when present*): Fe occurs only in traces. ~~The~~ most important element of the proteid molecule is the nitrogen. The human body needs nitrogen for the continuation of life, but it cannot use the nitrogen of the air or that in various other combinations such as we find in nitrites, etc. However, in the proteid molecule the nitrogen is present in a form which is utilizable by the body.

No definite knowledge has yet been secured regarding the constitutional formula or the molecular weight of proteid material. The molecular weight of egg albumin has been placed at about 15,000 and the formula for the crystallized product has been calculated as $C_{230}H_{380}N_{58}S_2O_{78}$. Many important and valuable investigations have been promoted recently on the subject of the constitution of the proteid molecule and our knowledge has been largely increased.

The proteids may be classified as follows:

I. SIMPLE PROTEIDS.

1. NATIVE SIMPLE PROTEIDS.

(a) **Albumins**—egg albumin, serum albumin and vegetable albumins.

(b) **Globulins**—*serum globulin, ovoglobulin, edestin* and other vegetable globulins.

(c) **Phospho-proteids (nucleo-albumins)**—*caseinogen* and *vitellin*.

2. DERIVED SIMPLE PROTEIDS.

(a) **Albuminates**—*acid albuminate* and *alkali albuminate*.

(b) **Proteoses (or albumoses)** and **peptones**—*proto-proteose, heteroproteose* and *deuteroproteose*; *amphopeptone* and *antipeptone*.

(c) **Coagulated Proteids**—*fibrin*, and the products of heat coagulation, etc.

II. COMPOUND PROTEIDS.

(a) **Glucoproteids**—*mucins* (from fluids and secretions); *mucoids, c. g., osseomucoid* and *tendomucoid*; *amyloid*.

(b) **Nucleo-proteids.**

(c) **Hæmoglobins.**

III. ALBUMINOIDS, ALBUMOIDS OR PROTEOIDS (PROTEID-LIKE BODIES).

(a) **Chondroalbumoid**—isolated from cartilage.

(b) **Collagen**—constituent of connective tissue and particularly abundant in *tendinous tissue*.

(c) **Elastin**—constituent of connective tissue and particularly abundant in *ligament*.

(d) **Gelatin**—product of the hydrolysis of collagen.

(e) **Keratin**—forms the major portion of *hair, hoof, horn*, etc.

(f) **Osseoalbumoid**—isolated from *bone*.

(g) **Reticulin**—found in fibers of *reticular tissue*.

GENERAL COLOR REACTIONS OF PROTEIDS.

These color reactions are due to a reaction between some one or more of the constituent radicals or groups of the complex proteid molecule and the chemical reagent or reagents

used in any given test. Not all proteids contain the same groups and for this reason the various color tests will yield reactions varying in intensity of color according to the nature of the groups contained in the particular proteid under examination. Various substances not proteids respond to certain of these color reactions and it is therefore essential to submit the material under examination to several tests before concluding definitely regarding its nature.

TECHNIQUE OF THE COLOR REACTIONS.

1. **Millon's Reaction.**—To 5 c.c. of a dilute egg albumin solution in a test-tube add a few drops of Millon's reagent. A white precipitate forms which turns red when heated. This test is a particularly satisfactory one for use on *solid* proteids, in which case the reagent is added directly to the solid substance and heat applied, which causes the substance to assume a red color.

The reaction is due to the presence of the hydroxy-phenyl group, $\text{—C}_6\text{H}_4\text{OH}$, in the proteid molecule and certain non-proteids such as tyrosin and phenol (carbolic acid) also respond to the reaction. The test is not a very satisfactory one for use in solutions containing salts, since the mercury of the Millon's reagent¹ is thus precipitated and the reagent rendered inert.

2. **Xanthoproteic Reaction.**—To 2–3 c.c. of egg albumin solution in a test-tube add concentrated HNO_3 . A white precipitate forms, which upon heating turns yellow and finally dissolves, imparting to the solution a yellow color. *Cool the solution* and carefully add NH_4OH *in excess*. Note that the yellow color deepens into an orange. This reaction is due to the presence in the proteid molecule of the phenyl group, with which the nitric acid forms certain nitro modifications. The

¹ Millon's reagent consists of mercury dissolved in nitric acid containing some nitrous acid. It is prepared by digesting one part (by weight) of mercury with two parts (by weight) of HNO_3 (sp. gr. 1.42) and diluting the resulting solution with two volumes of water.

test is not a satisfactory one for use in urinary examination because of the color of the end-reaction.

3. **Adamkiewicz Reaction.**—Thoroughly mix 1 volume of concentrated H_2SO_4 and 2 volumes of acetic acid in a test-tube, add a few drops of egg albumin solution and heat gently. A reddish-violet color is produced. Gelatin does not respond to this test. This reaction shows the presence of the tryptophan group (see next experiment). The test depends upon the presence of glyoxylic acid, $\text{CHO} \cdot \text{COOH}$ in the reagents. This is shown by the failure to secure a positive reaction when acetic acid free from glyoxylic acid is used.

4. **Hopkins-Cole Reaction.**—Place 1–2 c.c. of egg albumin solution and 3 c.c. of glyoxylic acid, $\text{CHO} \cdot \text{COOH}$, solution (Hopkins-Cole reagent¹) in a test-tube and mix thoroughly. In a second tube place 5 c.c. of concentrated sulphuric acid. Incline the tube containing the sulphuric acid and by means of a pipette allow the albumin-glyoxylic acid solution to flow *carefully* down the side. When stratified in this manner a reddish-violet color forms at the zone of contact of the two fluids. This color is due to the presence of the tryptophan group. Gelatin does not respond to this test. For formula for tryptophan see page 77.

5. **Biuret Test.**—To 2–3 c.c. of egg albumin solution in a test-tube add an equal volume of concentrated KOH solution, mix thoroughly, and add slowly a very dilute (2–5 drops in a test-tube of water) cupric sulphate solution until a purplish-violet or pinkish-violet color is produced. The depth of the color depends upon the nature of the proteid, proteoses and peptones giving a decided pink, while the color produced with gelatin is not far removed from a blue. This reaction is given by those bodies which contain two amino groups (CONH_2 , $\text{C}(\text{NH})\text{NH}_2$, CH_2NH_2 or CSNH_2) united by a C or N atom or joined together directly, therefore certain non-proteids may

¹Hopkins-Cole reagent is prepared as follows: To one liter of a saturated solution of oxalic acid add 60 grams of sodium amalgam and allow the mixture to stand until the evolution of gas ceases. Filter and dilute with 2–3 volumes of water.

also give a positive biuret reaction. Proteid material responds positively since there are two CONH_2 groups in the proteid molecule. These groups are found in a substance called *biuret* (see page 243) which may be formed by heating *urea* to 180°C .

6. Posner's Modification of the Biuret Test.—This test is particularly satisfactory for use on *dilute* proteid solutions, and is carried out as follows: To some dilute egg albumin in a test-tube add one-half its volume of KOH solution. Now hold the tube in an inclined position and allow some very dilute cupric sulphate solution, made as suggested on page 45 (5), to flow down the side, being especially careful to prevent the fluids from mixing. At the juncture of the two solutions the typical end-reaction of the biuret test should appear as a colored zone (see Biuret Test, page 45).

7. Liebermann's Reaction.—Add about 10 drops of *concentrated* egg albumin solution (or a little dry egg albumin) to about 5 c.c. of concentrated HCl in a test-tube. Boil until a pinkish-violet color results.

PRECIPITATION REACTIONS AND OTHER PROTEID TESTS.

There are three forms in which proteids may be precipitated *i. e.*, *unaltered*, as an *albuminate*, and as an *insoluble salt*. An instance of the precipitation in a *native* or *unaltered* condition is seen in the so-called *salting-out* experiments. Various salts, notably $(\text{NH}_4)_2\text{SO}_4$, ZnSO_4 , MgSO_4 , Na_2SO_4 and NaCl possess the power, when added in *solid form* to certain definite proteid solutions, of rendering the menstruum incapable of holding the proteid in solution, thereby causing the proteid to be precipitated or *salted-out* to use the common term. Mineral acids and alcohol also precipitate proteids *unaltered*. Proteids are precipitated as *albuminates* when treated with certain metallic salts, and precipitated as *insoluble salts* when certain weak organic acids are added to their solutions.

EXPERIMENTS.

1. **Influence of Concentrated Mineral Acids, Alkalis and Organic Acids.**—Prepare 5 test-tubes each containing 5 c.c. of concentrated egg albumin solution. To the first add concentrated H_2SO_4 , drop by drop, until an excess of the acid has been added. Note any changes which may occur in the solution. Allow the tube to stand for 24 hours and at the end of that period observe any alteration which may have taken place. Heat the tube and note any further change which may occur. Repeat the experiment in the 4 remaining tubes with concentrated HCl , concentrated HNO_3 , concentrated KOH and CH_3COOH . How do strong mineral acids, strong alkalis and strong organic acids differ in their action toward proteid solutions?

2. **Precipitation by Metallic Salts.**—Prepare 4 tubes each containing 2–3 c.c. of dilute egg albumin solution. To the first add *mercuric chloride*, drop by drop, until an excess of the reagent has been added, noting any changes which may occur. Repeat the experiment with *plumbic acetate*, *argentic nitrate*, *cupric sulphate*, *ferric chloride* and *barium chloride*.

Egg albumin is used as an antidote for lead or mercury poisoning. Why?

3. **Precipitation by Alkaloidal Reagents.**—Prepare 6 tubes each containing 2–3 c.c. of dilute egg albumin solution. To the first add *picric acid* drop by drop until an excess of the reagent has been added, noting any changes which may occur. Repeat the experiment with *trichloroacetic acid*, *tannic acid*, *phosphotungstic acid*, *phosphomolybdic acid* and *potassio-mercuric iodide*. Acidify with HCl before testing with the three last reagents.

4. **Heller's Ring Test.**—Place 5 c.c. of concentrated HNO_3 in a test-tube, incline the tube, and by means of a pipette allow the dilute albumin solution to flow *slowly* down the side.¹ The liquids should stratify with the formation of

¹An apparatus called the *albumoscope* has been devised for use in this test and has met with considerable favor.

a white zone of precipitated albumin at the point of juncture. This is a very delicate test and is further discussed on p. 290.

5. **Roberts' Ring Test.**—Place 5 c.c. of Roberts' reagent¹ in a test-tube, incline the tube, and by means of a pipette allow the albumin solution to flow *slowly* down the side. The liquids should stratify with the formation of a *white zone* of precipitated albumin at the point of juncture. This test is a modification of Heller's ring test and is rather more satisfactory. (See page 291.)

6. **Spiegler's Ring Test.**—Place 5 c.c. of Spiegler's reagent² in a test-tube, incline the tube, and by means of a pipette allow 5 c.c. of albumin solution, acidified with acetic acid, to flow *slowly* down the side. A white zone will form at the point of contact. This is an exceedingly delicate test, in fact, too delicate for ordinary clinical purposes, since it serves to detect albumin when present in the merest trace (1:250,000). This test is further discussed on page 291.

7. **Jolles' Reaction.**—Shake 5 c.c. of albumin solution with 1 c.c. of 30 per cent acetic acid and 4 c.c. of Jolles' reagent³ in a test-tube. A *white* precipitate of albumin should form. Care should be taken to use the correct amount of acetic acid. For further discussion of the test see page 292.

8. **Tanret's Test.**—To 5 c.c. of albumin solution in a test-tube add Tanret's reagent,⁴ drop by drop, until a turbidity or

¹ Roberts' reagent is composed of 1 volume of concentrated HNO_3 and 5 volumes of a saturated solution of MgSO_4 .

² Spiegler's reagent has the following composition:

Tartaric acid	20 grams.
Mercuric chloride	40 "
Glycerin	100 "
Distilled water	1000 "

³ Jolles' reagent has the following composition:

Succinic acid	40 grams.
Mercuric chloride	20 "
Sodium chloride	20 "
Distilled water	1000 "

⁴ Tanret's reagent is prepared as follows: Dissolve 1.35 gram of mercuric chloride in 25 c.c. of water, add to this solution 3.32 grams of potassium iodide dissolved in 25 c.c. of water, then make the total solution up to 60 c.c. with water and add 20 c.c. of glacial acetic acid to the combined solutions.

precipitate forms. This is an exceedingly delicate test. Sometimes the albumin solution is stratified upon the reagent as in Heller's or Roberts' ring tests.

9. **Sodium Chloride and Acetic Acid Test.**—Mix two volumes of albumin solution and 1 volume of a saturated solution of sodium chloride in a test-tube, acidify with acetic acid and heat to boiling. The production of a cloudiness or the formation of a precipitate indicates the presence of albumin.

10. **Acetic Acid and Potassium Ferrocyanide Test.**—To 5 c.c. of dilute egg albumin solution in a test-tube add 5–10 drops of acetic acid. Mix well, and add potassium ferrocyanide, *drop by drop*, until a precipitate forms. This test is very delicate.

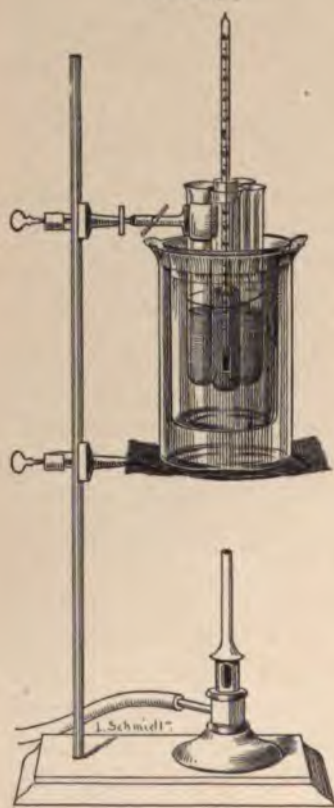
11. **Salting-out Experiments.**—(a) To 25 c.c. of egg albumin solution in a small beaker add *solid* $(\text{NH}_4)_2\text{SO}_4$ to the point of saturation, keeping the temperature of the solution below 40°C . Filter, test the precipitate by Millon's test and the filtrate by the biuret test. What are your conclusions? (b) Repeat the above experiment making the saturation with *solid* NaCl. How does this result differ from the result of the saturation with $(\text{NH}_4)_2\text{SO}_4$? Add 2–3 drops of acetic acid. What occurs? All proteids *except peptones* are precipitated by saturating their solutions with ammonium sulphate. *Globulins* are the only proteids precipitated by saturating with NaCl (see Globulins, page 53), unless the saturated solution is subsequently acidified, in which event all proteids *except peptones* are precipitated.

12. **Coagulation or Boiling Test.**—Heat 25 c.c. of dilute egg albumin solution to the boiling-point in a small evaporating dish. The albumin coagulates. Complete coagulation may be obtained by acidifying the solution with 3–5 drops of acetic acid¹ *at the boiling-point*. Test the coagulum by Millon's reaction. The acid is added to neutralize any possible alkalinity of the solution, and to dissolve any substances which are not albumin (see further discussion on page 292).

¹ Nitric acid is often used in place of acetic acid in this test. In case nitric acid is used, ordinarily 1–2 drops is sufficient.

13. **Coagulation Temperature.**—Prepare 4 test-tubes each containing 5 c.c. of *neutral* egg albumin solution. To the first add 1 drop of 0.2 per cent HCl, to the second add 1 drop of 0.5 per cent Na_2CO_3 solution, to the third add 1 drop of 10 per cent NaCl solution and leave the fourth neutral in reaction. Partly fill a beaker of medium size with water and place it within a second larger beaker which also contains water, the two vessels being separated by pieces of cork.

FIG. 19.

COAGULATION TEMPERATURE
APPARATUS.

Fasten the four test-tubes compactly together by means of a rubber band, lower them into the water of the inner beaker and suspend them, by means of a clamp attached to one of the tubes, in such a manner that the albumin solutions shall be midway between the upper and lower surfaces of the water. In one of the tubes place a thermometer with its bulb entirely beneath the surface of the albumin solution (Fig. 19). Gently heat the water in the beakers, noting carefully any changes which may occur in the albumin solutions and record the exact temperature at which these changes occur. The first appearance of an *opacity* in an albumin solution indicates the commencement of coagulation and the temperature at which this occurs should be recorded as the *coagulation temperature* for that particular albumin solution.

What is the order in which the four solutions coagulate?

Repeat the experiment adding to the first tube 1 drop of acetic acid, to the second 1 drop of concentrated KOH solution, to the third 2 drops of a 10 per cent NaCl solution and leave the fourth neutral as before.

acetic
base
Neutral
alkali

What is the order of coagulation here? Why?

14. **Precipitation by Alcohol.**—Prepare 3 test-tubes each containing about 10 c.c. of 95 per cent alcohol. To the first add one drop of 0.2 per cent HCl, to the second one drop of KOH solution and leave the third neutral in reaction. Add to each tube a few drops of egg albumin solution and note the results. What do you conclude from this experiment? Alcohol precipitates proteids unaltered but if allowed to remain under alcohol the proteid is coagulated. The "fixing" of tissues for histological examination by means of alcohol is an illustration of the application of this reaction.

15. **Preparation of Powdered Egg Albumin.**—This may be prepared as follows: Ordinary egg-white finely divided by means of scissors or a beater is treated with 4 volumes of water and filtered. The filtrate is evaporated on a water-bath at about 50° C. and the residue powdered in a mortar.

16. **Tests on Powdered Egg Albumin.**—With powdered albumin prepared as described above (by yourself or furnished by the instructor), try the following tests:

(a) *Solubility.*

(b) *Millon's Reaction.*

(c) *Hopkins-Cole Reaction.*—When used to detect the presence of proteid in solid form this reaction should be conducted as follows: Place 5 c.c. of concentrated H_2SO_4 in a test-tube and add carefully, by means of a pipette, 3–5 c.c. of Hopkins-Cole reagent. Introduce a small amount of the solid substance to be tested, agitate the tube slightly, and note that the suspended pieces assume a reddish-violet color, which is the characteristic end-reaction of the Hopkins-Cole test; later the solution will also assume the reddish-violet color.

(d) *Composition Test.*—Heat some of the powder in a test-tube in which is suspended a strip of moistened red litmus

paper and across the mouth of which is placed a piece of filter paper moistened with plumbic acetate solution. As the powder is heated it chars, indicating the presence of *carbon*; the fumes of ammonia are evolved, turning the red litmus paper blue and indicating the presence of *nitrogen* and *hydrogen*; the plumbic acetate paper is blackened, indicating the presence of *sulphur*, and the deposition of moisture on the side of the tube indicates the presence of *hydrogen*.

(c) Immerse a tube containing a little powdered egg albumin in boiling water for a few moments. Remove and test the solubility of the albumin according to the directions given under (a) above. It is still soluble. Why has it not been coagulated? Repeat the above experiments with powdered serum albumin and see how the results compare with those just obtained.

SULPHUR IN PROTEID.

Sulphur is believed to be present in two different forms in the proteid molecule. The first form, which is present in greatest amount, is that loosely combined with carbon and hydrogen. Sulphur in this form is variously termed *unoxidized*, *loosely combined*, *mercaptan* and *lead-blackening sulphur*. The second form is combined in a more stable manner with carbon and oxygen and is known as *oxidized* or *acid sulphur*.

TESTS FOR SULPHUR.

1. **Test for Loosely Combined Sulphur.**—To equal volumes of KOH and egg albumin solutions in a test-tube add 1–2 drops of plumbic acetate solution and boil the mixture. Loosely combined sulphur is indicated by a darkening of the solution, the color deepening into a black if sufficient sulphur is present. Write the reactions for this test.

2. **Test for Total Sulphur (Loosely Combined and Oxidized).**—Place the substance to be examined (powdered egg albumin) in a small porcelain crucible, add a suitable amount of solid fusion mixture (KOH and KNO_3 mixed in the pro-

portion 5:1) and heat carefully until a colorless mixture results. Cool, dissolve the cake in a little warm water and filter. Acidify the filtrate with HCl, heat it to the boiling-point and add a small amount of BaCl₂ solution. A white precipitate forms if sulphur is present. What is this precipitate?

GLOBULINS.

Globulins are simple proteids especially predominant in the vegetable kingdom. They are closely related to the albumins and in common with them give all the ordinary proteid tests. Globulins differ from the albumins in being insoluble in water. Most globulins are precipitated from their solutions by saturation with solid sodium chloride or magnesium sulphate. As a class they are much less stable than the albumins, a fact shown by the increasing difficulty with which a globulin dissolves during the course of successive reprecipitations.

We have used an albumin of animal origin (egg albumin) for all the proteid tests thus far, whereas the globulin to be studied will be prepared from a vegetable source. There being no essential difference between animal and vegetable proteids, the vegetable globulin we shall study may be taken as a true type of all globulins, both animal and vegetable.

EXPERIMENTS ON GLOBULIN.

Preparation of the Globulin.—Extract 20–30 grams (a handful) of crushed hemp seed with a 5 per cent solution of NaCl for one-half hour at 60° C. Filter while hot through a paper moistened with 5 per cent NaCl solution and allow the filtrate to cool *slowly*. The globulin is soluble in *hot* 5 per cent NaCl solution and is thus extracted from the hemp seed, but upon cooling this solution much of the globulin separates in crystalline form. This particular globulin is called *edestin*. It crystallizes in several different forms, chiefly octahedra (see Fig. 20, page 54). (The crystalline form of *excelsin*, a proteid obtained from the Brazil nut, is shown in Fig. 21, page 55. This vegetable proteid crystallizes in the form of hexagonal plates.) Filter off the *edestin* and make the follow-

ing tests on the crystalline body and on the filtrate which still contains some of the extracted globulin.

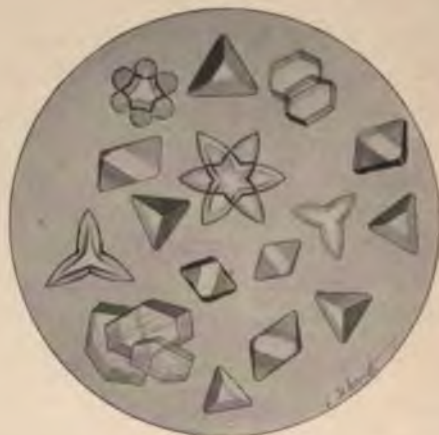
TESTS ON CRYSTALLIZED EDESTIN.—(1) *Microscopical examination* (Fig. 20, below).

(2) *Solubility*.—Try the solubility in the ordinary solvents (see page 4).

(3) *Millon's Reaction*.

(4) *Coagulation Test*.—Place a small amount of the globulin in a tube, add a little water and boil. Now add dilute HCl and note that the proteid no longer dissolves. It has been coagulated.

FIG. 20.



EDESTIN.

TESTS ON EDESTIN FILTRATE.—(1) *Influence of Proteid Precipitants*.—Try a few proteid precipitants such as *nitric acid*, *tannic acid*, *picric acid* and *mercuric chloride*.

(2) *Biuret Test*.

(3) *Coagulation Test*.—Boil some of the filtrate in a test-tube. What happens?

(4) *Saturation with Sodium Chloride*.—Saturate some of the filtrate with *solid NaCl*. How does this result differ from that obtained upon saturating egg albumin solution with solid NaCl?

(5) *Precipitation by Dilution.*—Dilute some of the filtrate with 10–15 volumes of water. Why does the globulin precipitate?

DERIVED SIMPLE PROTEIDS.

These bodies are obtained from native simple proteids by various means, *e. g.*, through the action of acids, alkalis, heat or enzymes, the method of treatment to which the native proteid is subjected depending upon the particular class of derived proteid desired. These modified bodies are classified as albuminates, proteoses (or albumoses), peptones and coagulated proteids.

Albuminates.

The albuminates are derived proteids and are produced by the action of acids or alkalis upon the native simple proteids, albumins and globulins. There are two classes of albuminates,

FIG. 21.



EXCELSIN, THE PROTEID OF THE BRAZIL NUT.
(Drawn from crystals furnished by Dr. Thomas B. Osborne, New Haven, Conn.)

i. e., acid albuminate and alkali albuminate. They differ from the native simple proteids principally in being insoluble in NaCl solution and in not being coagulated *except when sus-*

pended in neutral fluids. Both forms of albuminate are precipitated upon the neutralization of their solutions. They are precipitated by saturation with $(\text{NH}_4)_2\text{SO}_4$, and by saturation with NaCl also if they are dissolved in an *acid* solution. Acid albuminate contains a higher percentage of nitrogen and sulphur than the alkali albuminate from the same source since some of the nitrogen and sulphur of the original proteid is liberated in the formation of the latter. Because of this fact it is impossible to transform an alkali albuminate into an acid albuminate, while it is possible to reverse the process and transform the acid albuminate into the alkali modification.

ACID ALBUMINATE.

Preparation.—Take 25 grams of hashed lean beef, washed free from the major portion of blood and inorganic matter, and place it in a medium-sized beaker with 100 c.c. of 0.2 per cent HCl . Place it on a boiling water-bath for one-half hour, filter, *cool* and divide the filtrate into two parts. Neutralize the *first part* with *dilute* KOH solution, filter off the precipitate of *acid albuminate* and make the following tests:

(1) *Solubility.*—Solubility in the ordinary solvents (see page 4).

(2) *Millon's Reaction.*

(3) *Coagulation Test.*—Suspend a little of the albuminate in water (neutral solution) and heat to boiling for a few moments. Now add 1–2 drops of KOH solution to the water and see if the albuminate is still soluble in dilute alkali. What is the result and why?

(4) *Test for Loosely Combined Sulphur* (see page 52).

Subject the *second part* of the solution to the following tests:

(1) *Coagulation Test.*—Heat some of the solution to boiling in a test-tube. Does it coagulate?

(2) *Biuret Test.*

(3) *Influence of Proteid Precipitants.*—Try a few proteid precipitants such as *picric acid* and *mercuric chloride*. How

do the results obtained compare with those from the experiments on egg albumin? (See page 47.)

ALKALI ALBUMINATE.

Preparation.—Carefully separate the white from the yolk of a hen's egg and place the former in an evaporating dish. Add concentrated KOH solution, *drop by drop*, stirring continuously. The mass gradually thickens and finally assumes the consistency of jelly. This is *solid alkali albuminate* or "Lieberkühn's jelly." Do not add an excess of KOH or the jelly will dissolve. Cut it into small pieces, place a cloth or wire gauze over the dish and by means of running water wash the pieces free from adherent alkali. Now add a small amount of water, which forms a weak alkaline solution with the alkali within the pieces, and dissolve the jelly by gentle heat. *Cool* the solution and divide it into two parts. Proceed as follows with the *first part*: Neutralize with *dilute* HCl, noting the odor of the liberated H_2S , as the alkali albuminate precipitates. Filter off the precipitate and test as for acid albuminate, page 56, noting particularly the sulphur test. How does this test compare with that given by the acid albuminate? Make tests on the *second part* of the solution the same as for acid albuminate, page 56.

Proteoses (or Albumoses) and Peptones.

Proteoses are intermediate products in the digestion of proteids by proteolytic enzymes, as well as in the decomposition of proteids by hydrolysis and the putrefaction of proteids through the action of bacteria. Peptones are formed after the proteoses and are the last products of the above mentioned processes which still possess true proteid characteristics. In other words, the proteid nature of the end-products of the cleavage of the proteid molecule ceases with the peptones, and the simpler bodies formed from peptones are bodies of a different type (see page 65).

There are several proteoses (protoproteose, heteroproteose and deuteroproteose), and at least two peptones (amphopep-

tone and antipeptone), which result from proteolysis. The differentiation of the various proteoses and peptones at present in use is rather unsatisfactory. These compounds are classified according to their varying solubilities, especially in $(\text{NH}_4)_2\text{SO}_4$ solutions of different strengths. The exact differences in composition between the various members of the group remains to be more accurately established. Because of the difficulty attending the separation of these bodies, pure proteose and peptone are not easy to procure. The so-called peptones sold commercially contain a large amount of proteose. As a class the proteoses and peptones are very soluble, diffusible bodies which are non-coagulable by heat. *Peptones differ from proteoses in being more diffusible, non-precipitable by $(\text{NH}_4)_2\text{SO}_4$, and by their failure to give any reaction with potassium ferrocyanide and acetic acid, potassio-mercuric iodide and HCl, picric acid, and trichloroacetic acid.* The so-called *primary proteoses* are precipitated by HNO_3 and are the only members of the proteose-peptone group which are so precipitated.

Some of the more general characteristics of the proteose-peptone group may be noted by making the following simple tests on a proteose-peptone powder:

(1) *Solubility*.—Solubility in the ordinary solvents (see page 4).

(2) *Millon's Reaction*.

Dissolve a little of the powder in water and test the solution as follows:

(1) *Precipitation by Picric Acid*.—To 5 c.c. of proteose-peptone solution in a test-tube add picric acid until a permanent precipitate forms. The precipitate disappears on heating and returns on cooling.

(2) *Precipitation by a Mineral Acid*.—Try the precipitation by nitric acid.

(3) *Coagulation Test*.—Heat a little proteose-peptone solution to boiling. Does it coagulate like the other simple proteids studied?

SEPARATION OF PROTEOSES AND PEPTONES.

Place 50 c.c. of proteose-peptone solution in an evaporating dish or casserole, and *half-saturate* it with $(\text{NH}_4)_2\text{SO}_4$ solution, which may be accomplished by adding an equal volume of *saturated* $(\text{NH}_4)_2\text{SO}_4$ solution. At this point note the appearance of a precipitate of the *primary proteoses* (proteose and heteroproteose). Now heat the half-saturated solution and its suspended precipitate to boiling and *saturate* the solution with *solid* $(\text{NH}_4)_2\text{SO}_4$. At full saturation the *secondary proteoses* (deuteroproteoses) are precipitated. The peptones remain in solution.

Proceed as follows with the precipitate of proteoses: Collect the sticky precipitate on a rubber-tipped stirring rod or remove it by means of a watch glass to a small evaporating dish and dissolve it in a little water. To remove the $(\text{NH}_4)_2\text{SO}_4$, which adhered to the precipitate and is now in solution, add BaCO_3 , boil, and filter off the precipitate of BaSO_4 . Concentrate the proteose solution to a small volume¹ and make the following tests:

(1) *Biuret Test*.

(2) *Precipitation by HNO_3* .—What would a precipitate at this point indicate?

(3) *Precipitation by Trichloroacetic Acid*.—This precipitate dissolves on heating and returns on cooling.

(4) *Precipitation by Picric Acid*.—This precipitate also disappears on heating and returns on cooling.

(5) *Precipitation by Potassio-mercuric Iodide and HCl* .

(6) *Coagulation Test*.—Boil a little in a test-tube. Does it coagulate?

(7) *Acetic Acid and Potassium Ferrocyanide Test*.

The solution containing the peptones should be cooled and

¹ If the proteoses are desired in powder form, this concentrated proteose solution may now be precipitated by alcohol, and this precipitate, after being washed with absolute alcohol and with ether, may be dried and powdered.

filtered, and the $(\text{NH}_4)_2\text{SO}_4$ in solution removed by boiling with BaCO_3 as described on page 59. After filtering off the BaSO_4 precipitate, concentrate the peptone filtrate to a small volume¹ and repeat the tests as given under the proteose solution, page 59. In the biuret test the solution should be made very strongly alkaline with *solid* KOH.

Coagulated Proteids.

These derived proteids are produced from unaltered proteid materials by heat, by long standing under alcohol, or by the continuous movement of their solutions such as that produced by rapid stirring or shaking. In particular instances, such as the formation of fibrin from fibrinogen (see page 157), the coagulation may be produced by ferment action. Ordinary soluble proteids after having been transformed into the coagulated modification are no longer soluble in the ordinary solvents. Upon being heated in the presence of strong acids or alkalis, coagulated proteids are converted into albuminates.

Many proteids coagulate at an approximately fixed temperature under definite conditions (see page 50). This characteristic may be applied to separate different coagulable proteids from the same solution by fractional coagulation. The coagulation temperature frequently may serve in a measure to identify proteids in a manner similar to the melting-point or boiling-point of many other organic substances. The separation of proteids by fractional coagulation thus is analogous to the separation of volatile substances by means of *fractional distillation*. The nature of the process involved in the coagulation of proteids by heat is not well understood, but it is probable that in addition to the altered arrangement of the component atoms in the molecule, there is a mild hydrolysis which is accompanied by the liberation of minute amounts of hydrogen, nitrogen and sulphur. The presence of a neutral salt or a trace of a mineral acid may facilitate the coagulation of a proteid solution (see page 50),

¹ See note on preparation of proteose powder, page 59.

whereas any appreciable amount of acid or alkali will retard or entirely prevent such coagulation.

EXPERIMENTS ON COAGULATED PROTEID.

Ordinary coagulated egg-white may be used in the following tests:

1. **Solubility.**—Try the solubility of *small* pieces of the coagulated proteid in each of the ordinary solvents (see page 4).

2. **Millon's Reaction.**

3. **Xanthoproteic Reaction.**—*Partly* dissolve a medium-sized piece of the proteid in concentrated HNO_3 . Cool the solution and add an excess of NH_4OH . Both the proteid solution and the undissolved proteid will be colored orange.

4. **Biuret Test.**—*Partly* dissolve a medium-sized piece of the proteid in concentrated KOH solution. If the proper dilution of CuSO_4 solution is now added the white coagulated proteid, as well as the proteid solution, will assume the characteristic purplish-violet color.

5. **Hopkins-Cole Reaction.**—Conduct this test according to the modification given on page 51.

COMPOUND PROTEIDS.

Compound proteids consist of a simple proteid combined with some non-proteid material, and they are named according to the nature of this combining body. Thus we have *glucoproteids*, *nucleoproteids* and *hæmoglobins* as three classes of compound proteids.

The *glucoproteids* yield, upon decomposition, proteid and carbohydrate derivatives, notably glucosamine, $\text{CH}_2\text{OH} \cdot (\text{CHOH})_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{CHO}$, and galactosamine, $\text{OHCH}_2 \cdot (\text{CHOH})_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{CHO}$. The principal glucoproteids are *mucoids*, *mucins* and *chondroproteids*. By the term *mucoïd* we may designate those glucoproteids which occur in tissues, such as *tendomucoid* from tendinous tissue and

osseomucoid from bone. The elementary composition of these typical mucoids is as follows:

	N.	S.	C.	H.	O.
Tendomucoid	11.75	2.33	48.76	6.53	30.60 (Chittenden and Gies)
Osseomucoid	12.22	2.32	47.43	6.63	31.40

The term *mucins* may be said to include those forms of glucoproteids which occur in the secretions and fluids of the body. Chondroproteids are so named because *chondromucoid*, the principal member of the group, is derived from cartilage (chondrigen). *Amyloid*, which appears pathologically in the spleen, liver and kidneys is also a chondroproteid.

The *nucleoproteids* occur principally in animal and vegetable cells, and following the destruction of these cells they are found in the fluids of the body. These proteids are discharged into the tissue fluids by the activity or disintegration of cells. Combined with the simple proteid in the nucleoproteid molecule we find *nucleic acid*, a body which contains phosphorus and which yields *purin bases* upon decomposition. The so-called *nucleins* are formed in the gastric digestion of nucleoproteids.

The *hemoglobins* are those compound proteids which are composed of a simple proteid and a pigment. The *hemoglobin* of the blood (see page 156) upon decomposition yields a proteid termed *globin* and a modified pigment called *hematin*.

For experiments upon a compound proteid see page 199.

ALBUMINOIDS, ALBUMOIDS OR PROTEOIDS.

These bodies are closely related in character to the proteids, from which class of substances they are derived. They differ ordinarily from true proteids in the character of their decomposition products, in being very resistant to the ordinary proteid solvents, and in being unable alone to support life. They generally occur in an insoluble form in some portion of the animal organism. The albuminoids may be divided into several classes such as *keratins*, *elastins*, *collagens*, *gelatins* and

skeletins, and in general the members of each group differ fundamentally in certain characteristics from the members of any other group. For discussion of and experiments on each of the several groups see the chapter on Epithelial and Connective Tissues, pages 197 to 205.

REVIEW OF PROTEIDS.

In order to facilitate the student's review of the proteids, the preparation of a chart similar to the appended model is recommended. The signs + and — may be conveniently used to indicate positive and negative reactions.

MODEL CHART FOR REVIEW PURPOSES.

Proteid.	Solubility.					Precipitation Tests.					Solting-out Tests.		Diffusion.	Coagulation by Heat.			
	Water.	10 % NaCl.	0.2 % HCl.	0.5 % Na_2CO_3 .	Conc. HCl.	Conc. KOH.	Proteid Color Tests.	Mineral Acid (HNO_3).	Metallic Salt (HgCl_2).	Alcohol.	Pot. Ferrocyanide + Acetic Acid.	Potassio-mercuric Iodide + HCl.			Picric Acid.	Trichloroacetic Acid.	(NH_4 SO_4).
Albumin.																	
Globulin.																	
Acid albuminate.																	
Alkali albuminate.																	
Proteose.																	
Peptone.																	
Coagulated proteid.																	

"UNKNOWN" MIXTURES AND SOLUTIONS OF PROTEIDS.

At this point the student's knowledge of the characteristics of the various proteids studied will be tested by requiring him to examine several "unknown" proteid mixtures or solutions and make full report upon the same. The scheme given on page 64 may be used in this examination.

SCHEME FOR THE DETECTION OF PROTEIDS.

If the solution is acid or alkaline it should be *approximately* neutralized.
 If a precipitate forms it should be filtered off. The neutralization need not necessarily proceed until an exact neutral reaction is obtained but should cease at the point where the largest precipitate is secured.

Precipitate indicates.

Acid albuminate, if the unknown solution was acid in reaction (Test the albuminate by protein color tests.)

Alkali albuminate, *Alkali albuminate*, *mucin (mucoid) or phosphoprotein (nucleo-albumin or nucleo-proteid)* if the unknown solution was alkaline in reaction.

Alkali albuminate, *Phosphoprotein* if the precipitate is *nucleo-albumin* or *nucleo-proteid* if the precipitate is *acidly* soluble in an excess of acid. (Test the albuminate by protein color tests.) of acid. (Test the precipitate by protein color tests. Also test the precipitate for phosphorus by test on page 223.)

Mucin or mucoid, *albumin or globulin* if the precipitate is soluble with difficulty in an excess of acid. Hydrolyze some of the precipitate and test by Fehling's solution. See page 199. *Albumin and globulin* if the precipitate is soluble with some of the unknown solution with $(\text{NH}_4)_2\text{SO}_4$. (By adding an equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$ solution.) If a precipitate forms, filter it off.

Filtrate. *Half-saturate* with $(\text{NH}_4)_2\text{SO}_4$.

Precipitate indicates *primary protease*. Filtrate. *Saturate* *hot* with $(\text{NH}_4)_2\text{SO}_4$.

Precipitate indicates *secondary protease or gelatin*. Filtrate. Cool and filter off the $(\text{NH}_4)_2\text{SO}_4$.

Dissolve the precipitate in warm water and saturate this neutral solution with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ solid KOH.)

Precipitate indicates *gelatin*. (Try any tests given on page 500.) Filtrate may contain *secondary protease*. (Try any tests given on page 500.)

Heat to boiling.

Coagulation indicates *albumin*. No coagulation indicates *gelatin*.

¹ We may also differentiate between gelatin and protease by means of the Hopkins-Cole reaction (see page 43). A positive reaction here would indicate protease and a negative reaction would indicate gelatin.

CHAPTER IV.

DECOMPOSITION PRODUCTS OF PROTEIDS.

Although various physico-chemical considerations indicate that the proteid molecule is very large, approaching a molecular weight of 15,000 (T. B. Osborne), more definite statements on this point cannot be made at the present time; nor can any definite constitutional formula yet be assigned to any of the proteid substances. Notwithstanding this, by a study of the decomposition products of proteids much has been learned regarding the inner structure of the complex molecule; our knowledge in this direction has recently been greatly advanced. Decomposition may be brought about by *oxidation* or by *hydrolysis*, the end-products of the decomposition varying more or less in character, according to the nature of the process. Oxidation is ordinarily facilitated by the use of such oxidizing agents as potassium permanganate, hydrogen peroxide or bromine, while hydrolysis is brought about by acids, alkalis or superheated steam, and in digestion by the action of the proteolytic enzymes. Among the decomposition products of proteids are *proteoses*, *peptones*, *carbon dioxide*, *ammonia*, *hydrogen sulphide*, *amines*,¹ *amides*,² *tryptophan*, *mono-amino acids* (such as *leucin*, *tyrosin*, *cystin*, *aspartic acid*, *glutamic acid*, *glycocoll*, *alanin*, *phenylalanin*, *amino-valerianic acid*, *prolin*, *oxyprolin*, *serin*) and *di-amino acids*, such as *arginin*, *lysin* and *histidin*.

Proteids may also be decomposed by putrefactive bacteria with the formation of such bodies as phenol, para-cresol, indol, skatol, etc. (see page 129).

¹ An *amine* is a body formed after the type of ammonia, one or more hydrogen atoms being replaced by *hydrocarbon radicals*.

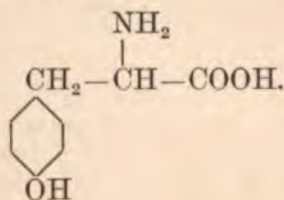
² An *amide* is a body formed after the type of ammonia, one or more hydrogen atoms being replaced by *organic acid radicals*, i. e., that portion of the acid left after removing the hydroxyl group.

For the benefit of those especially interested in such matters a photograph of the Fischer apparatus (Fig. 22, page 67) used in the fractional distillation, *in vacuo*, of the esters of the decomposition products of the proteids, as well as micro-photographs and drawings of preparations of several of these decomposition products (Figs. — to —, pp. — to —) are introduced. For the preparations and the photograph of the apparatus the author is indebted to Dr. T. B. Osborne, of New Haven, Conn. These drawings and photographs are not introduced at this point through any preconceived notion that the student will derive any practical benefit therefrom, but are rather inserted with the idea of giving him a graphical illustration of the magnitude of the proteid molecule, and with the hope that they may perhaps act as a stimulus, which will cause him to desire a more extended knowledge of the science of physiological chemistry, and, in particular, of that important class of substances, the proteids. The reproduction of the crystalline form of some of the more recent of the products may be of interest to those viewing the field of physiological chemistry from other than the student's aspect.

Any extended discussion of the various decomposition products being out of place in a book of this character, we will simply make a few general statements in connection with the more important products.

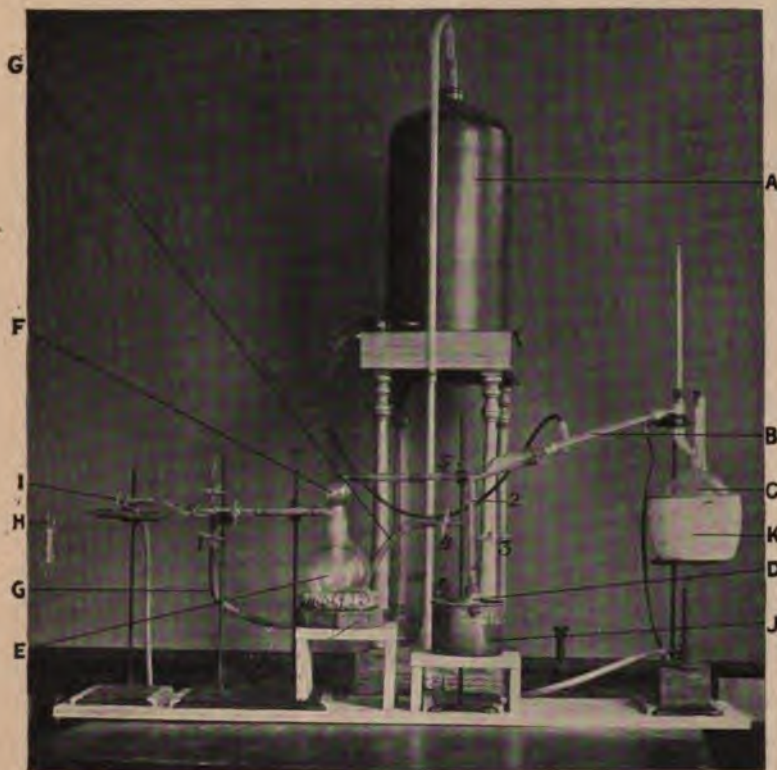
DISCUSSION OF THE PRODUCTS.

Tyrosin, $C_9H_{11}NO_3$.—Tyrosin, one of the most important end-products of proteid decomposition, is the amino acid, *p-oxyphenyl- α -amino-propionic acid*. It has the following formula:



Tyrosin occurs in conspicuous amounts as an end-product of the pancreatic digestion of proteids (see page 107), and is generally accompanied by leucin. It does not occur, however,

FIG. 22.



FISCHER APPARATUS.

Reproduced from a photograph made by Prof. E. T. Reichert, of the University of Pennsylvania. The negative was furnished by Dr. T. B. Osborne, of New Haven, Conn.

A, Tank into which freezing mixture is pumped and from which it flows through the condenser, *B*; *C*, flask from which the esters are distilled, the distillate being collected in *D*; *E*, a Dewar flask containing liquid air serving as a cooler for condensing tube *F*; *G* and *G'*, tubes leading to the Geryck pump by which the vacuum is maintained; *I*, tube leading to a McLeod gauge (not shown in figure); *J*, a bath containing freezing mixture in which the receiver *D* is immersed; *K*, a bath of water during the first part of the distillation and of oil during the last part of the process; *t-5*, stop cocks which permit the cutting out of different parts of the apparatus as the procedure demands.

as an end-product of the decomposition of gelatin. Tyrosin and leucin have the power of forming salts with copper. Since the tyrosin salt is much less soluble than the leucin salt, this forms the basis for a method of separating these bodies.

FIG. 23.

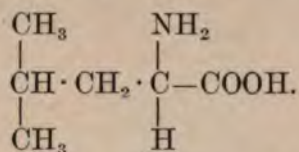


TYROSIN.

Tyrosin is found in old cheese, and derives its name from this fact. It crystallizes in tufts, sheaves or balls of fine needles, which melt at 295° C. and are rather insoluble in cold (1-2454) or boiling (1-154) water. It is soluble in alkalis, ammonia or mineral acids, and less easily soluble in acetic acid or hot 95 per cent alcohol. Tyrosin responds to Millon's reaction, thus showing the presence of the hydroxy-phenyl group, but gives no other proteid test. In severe cases of typhoid fever and smallpox, in acute yellow atrophy of the liver, and in acute phosphorus poisoning, tyrosin has been found in the urine. Tyrosin crystals obtained as a decomposition product of the proteid *gliadin* are shown in Fig. 23, above.

Leucin, $C_6H_{13}NO_2$.—Leucin is an important end-product of the decomposition of proteid material, and was the first of these products to be discovered (1818). It is an amino acid,

α-amino-isobutyl-acetic acid, and therefore has the following formula :



It is present *normally* in the pancreas, thymus, thyroid, spleen, brain, liver, kidneys and salivary glands. It has been found *pathologically* in the urine (in acute yellow atrophy of the liver, in acute phosphorus poisoning and in severe cases of typhoid fever and smallpox), and in the liver, blood and pus.

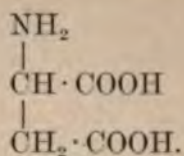
FIG. 24.



LEUCIN.

Pure leucin crystallizes in thin, white hexagonal plates. Crystals of pure leucin, obtained as a decomposition product of the proteid *gliadin*, are reproduced in Fig. 24. Impure leucin is a slightly refractive substance, which generally crystallizes in balls having a radial structure or in aggregations of spherical bodies, Fig. 104, page 326. It is rather easily soluble in water (46 parts), alcohol, alkalis, ammonia and acids. On heating to 170° C., leucin sublimes with the formation of carbon dioxide, ammonia and amylamine. In aqueous solutions leucin is *lævorotatory*, whereas in acid or alkaline solutions it is *dextrorotatory*.

Aspartic Acid, $C_4H_7NO_4$.—Chemically, aspartic acid is *amino-succinic acid* and has the following structural formula:



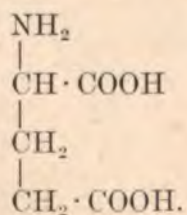
The amide of aspartic acid, *asparagin*, is very widely distributed in the vegetable kingdom. The crystalline form of aspartic acid, as obtained from the proteid *glutenin*, is exhibited in Fig. 25.

FIG. 25.



ASPARTIC ACID.

Glutamic Acid, $C_5H_9NO_4$.—This acid is *α -amino-normal-glutaric acid* and as such bears the following graphic formula:



Upon hydrolyzing the wheat proteid gliadin with hydrochloric acid, Osborne and Harris obtained a yield of 37 per cent of glutamic acid. This is the largest amount of an amino

FIG. 26.

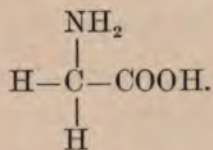


GLUTAMIC ACID.

Reproduced from a micro-photograph made by Prof. E. T. Reichert, of the University of Pennsylvania.

acid yet obtained as a decomposition product of any proteid substance. Crystals of glutamic acid, obtained from the proteid *gliadin*, are reproduced in Fig. 26.

Glycocoll, $C_2H_5NO_2$.—Glycocoll, or *amino acetic acid*, is the simplest of the amino acids and has the following formula:



It was the second of the decomposition products of proteids to be discovered, being preceded only by leucin. In distinction from the greater number of the amino acids, glycocoll may be determined quantitatively very accurately. Hippuric acid may be formed synthetically through a union of glycocoll and ben-

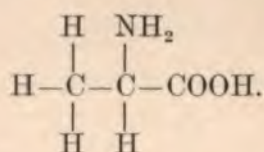
zoic acid (see page 263). This method is not used very extensively at present, however, having been replaced by Fischer's method. Glycocoll, ingested in small amount, is excreted in the urine as urea, whereas if administered in excess it appears in part unchanged in the urine. The crystalline form of glycocoll ester hydrochloride, resulting from the decomposition of the proteid *glutenin*, is shown in Fig. 27.

FIG. 27.



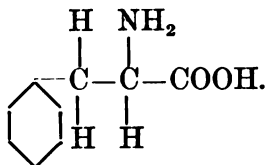
GLYCOCOLL ESTER HYDROCHLORIDE.

Alanin, $C_3H_7NO_2$.—From a chemical view-point this decomposition product is *α-amino-propionic acid*, and as such it may be represented structurally as follows:



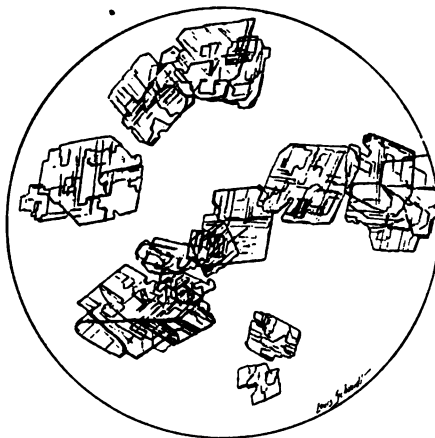
Obtained from proteid substances, alanin is dextrorotatory, is very easily soluble in water, and possesses a sweet taste. Tyrosin, phenylalanin, cystin and serin are derivatives of alanin.

Phenylalanin, $C_9H_{11}NO_2$.—This product is *phenyl- α -amino-propionic acid*, and may be represented graphically as follows:



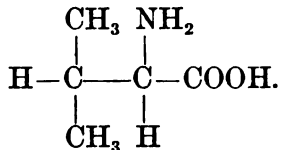
Phenylalanin is not so soluble as alanin, and possesses a sweet taste. The yield of this body from the decomposition of proteids is frequently greater than the yield of tyrosin. The crystalline form of phenylalanin obtained from the proteid *gliadin* is shown in Fig. 28.

FIG. 28.



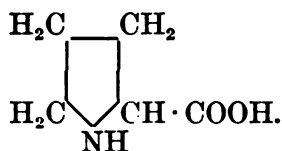
PHENYLALANIN.

Amino-valerianic Acid, $C_6H_{11}NO_2$.—This acid is probably *α -amino-isovalerianic acid*, and as such bears the following formula:



It closely resembles leucin in many of its properties, and for this reason is difficult to identify in the presence of leucin. It is quite readily soluble in water and is dextrorotatory.

Prolin, $C_5H_9NO_2$.—Chemically, prolin is *α -pyrrolidine-carboxylic acid* and therefore possesses the following graphic structure:



Prolin was first obtained as a decomposition product of **casein**, is *laevorotatory* and possesses a very sweet taste. The **crystalline form of *laevo*- α -prolin** is shown in Fig. 29, and the **copper**

FIG. 29.

LEVO- α -PROLIN.

salt of prolin is represented by a micro-photograph in Fig. 30, page 75. Both were obtained from the proteid *gliadin*. The crystals of the copper salt possess a faint bluish tinge.

Serin, $C_3H_7NO_3$.—Serin, from a chemical standpoint, is *α -amino- β -hydroxy-propionic acid* and possesses the following structural formula:

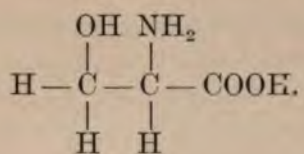


FIG. 30.



COPPER SALT OF PROLIN.

Reproduced from a micro-photograph made by Prof. E. T. Reichert, of the University of Pennsylvania.

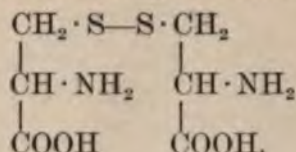
FIG. 31.



SERIN.

This product is inactive, possesses a sweet taste and is quite readily soluble in hot water. Serin crystals, obtained as a decomposition product of the proteid *gliadin*, are shown in Fig. 31, page 75.

Cystin, $C_6H_{12}O_4N_2S_2$.—Friedmann has recently shown cystin to possess the following structural formula:



Cystin is the principal sulphur-containing body obtained from the decomposition of proteid substances. It is obtained in greatest amount as a decomposition product of such keratin-containing tissues as horn, hoof and hair. Cystin occurs in

FIG. 32.



CYSTIN.

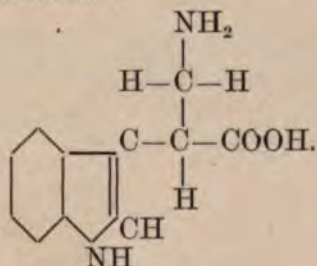
small amount in normal urine and is greatly increased in quantity under certain pathological conditions. It crystallizes in the form of hexagonal plates which are thin and colorless; crystals obtained from the decomposition of the proteid *gliadin* are shown in Fig. 32. Cystin is soluble in alkalis,

ammonia, oxalic acid solution and mineral acids but practically insoluble in water, acetic acid, alcohol and ether. It is lævorotatory.

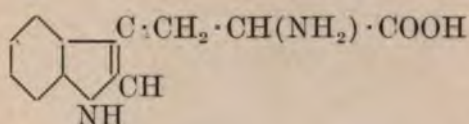
It has recently been claimed that cystin occurs in two forms, *i. e.*, stone-cystin and proteid-cystin and that these two forms are distinct in their properties. This view is incorrect.

For a discussion of cystin sediments in urine see Chapter XIX.

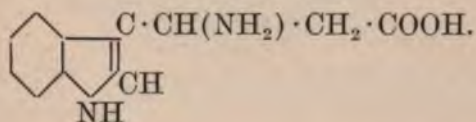
Tryptophan, $C_{11}H_{12}N_2O_2$.—According to Ellinger, tryptophan is *indol-amino-propionic acid*. Until very recently this investigator thought the following was the correct structural formula of this substance:



Further investigation by him, however, has shown this view to be incorrect. He now proposes the two formulas which follow and expects further study will show definitely which one correctly represents the structure of tryptophan:



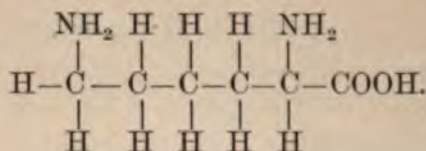
or



Tryptophan is the *mother-substance of indol* and its presence in proteid substances may be shown by means of the Adam-

kiewicz reaction or the Hopkins-Cole reaction (see p. 45). It may be detected in a pancreatic digestion mixture through its property of giving a violet color-reaction with bromine water.

Lysin, $C_6H_{14}N_2O_2$.—The three bodies, lysin, arginin and histidin, are frequently classed together as the *hexone bases*. Lysin was the first of the bases discovered. It is α - ϵ -diamino-caproic acid and hence possesses the following structure:



It is dextrorotatory and is found in largest amount in casein and gelatin. It is the mother-substance of cadaverin and has

FIG. 33.

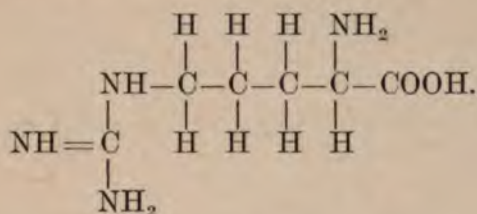


LYSIN PICRATE.

never been obtained in crystalline form. The picrate of lysin may be crystallized, however; the crystals of this body, obtained from the proteid *legumin*, are shown in Fig. 33.

Arginin, $C_6H_{14}N_4O_2$.—Arginin is the most widely distributed of the decomposition products of the proteids.

Every proteid so far subjected to decomposition has yielded this body among the products. Because of this fact, some investigators consider arginin to be the nucleus of the proteid molecule. Chemically, arginin is *guanidin- α -amino-valerianic acid* and possesses the following structural formula :



It is claimed that in the ordinary metabolic activities of the animal body arginin gives rise to urea.

Histidin, $\text{C}_6\text{H}_9\text{N}_3\text{O}_2$.—This body occurs most abundantly as a decomposition product of globin, the proteid constituent

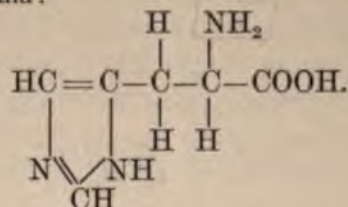
FIG. 34.



HISTIDIN HYDROCHLORIDE.

of hæmoglobin. The free base is lævorotatory, whereas the salts of histidin are dextro-rotatory. Histidin is now believed

to be α -amino- β -imido-azol-propionic acid with the following structural formula:



Crystals of histidin hydrochloride are shown in Fig. 34, p. 79.

EXPERIMENTS.

While the ordinary courses in physiological chemistry preclude any extended study of the decomposition products of proteids, the manipulation of a simple decomposition and the subsequent isolation and study of a few of the products most easily and quickly obtained will not be without interest. To this end the student may use the following decomposition procedure: Treat the proteid in a large flask with water containing 3-5 per cent of H_2SO_4 and place it on a water-bath until the proteid material has been decomposed and there remains a fine, fluffy, insoluble residue. Filter off this residue and neutralize the filtrate with $\text{Ba}(\text{OH})_2$ and BaCO_3 . Filter off the precipitate of BaSO_4 which forms and when certain that the fluid is neutral or faintly acid,¹ concentrate (first on a wire gauze and later on a water-bath) to a syrup. This syrup contains the end-products of the decomposition of the proteid, among which are *proteoses*, *peptones*, *tyrosin*, *leucin*, etc. Add 95 per cent alcohol slowly to the warm syrup until no more precipitate forms, stirring continuously with a glass rod. This precipitate consists of proteoses and peptones. Gather the sticky precipitate on the rod or the sides of the dish and, after warming the solution gently for a few moments, filter it through a filter paper which has not been previously moistened. After dissolving the precipitate of proteoses and pep-

¹ If the solution is alkaline in reaction at this point, the amino acids will be broken down and ammonia will be evolved.

tones in water¹ the solution may be treated according to the method separation given on page 59.

The leucin and tyrosin, etc., are in solution in the warm alcoholic filtrate. Concentrate this filtrate on the water-bath to a thin syrup, transfer it to a beaker, and allow it to stand over night in a cool place for crystallization. The tyrosin first crystallizes (Fig. 23, page 68), followed later by the formation of characteristic crystals of impure leucin (see Chapter XIX). After examining these crystals under the microscope, strain off the crystalline material through fine muslin, heat it gently in a little water to dissolve the leucin (the tyrosin will be practically insoluble) and filter. Concentrate the filtrate and allow it to stand in a cool place over night for the crude leucin to crystallize. Filter off the crystals and use them in the tests for leucin given on page 82. The crystals of tyrosin remaining on the paper from the first filtration may be used in the tests for tyrosin as given below. If desired, the tyrosin and leucin may be purified by recrystallizing in the usual manner. Habermann has suggested a method of separating leucin and tyrosin by means of glacial acetic acid.

EXPERIMENTS ON TYROSIN.

Make the following tests with the tyrosin crystals already prepared or upon some pure tyrosin furnished by the instructor.

1. **Microscopical Examination.**—Place a minute crystal of tyrosin on a slide, add a drop of water, cover with a cover glass, and examine microscopically. Now run more water under the cover glass and warm in a bunsen flame until the tyrosin has dissolved. Allow the solution to cool *slowly* then examine again microscopically and compare the crystals with those shown in Fig. 23, page 68.

¹At this point the aqueous solution of the proteoses and peptones may be filtered to remove any BaSO₄ which may still remain. Tyrosin crystals will also be found here, since it is less soluble than the leucin and may adhere to the proteose-peptone precipitate. Add the crystals of tyrosin to the warm alcohol filtrate.

2. **Solubility.**—Try the solubility of very *small amounts* of tyrosin in cold and hot water, cold and hot 95 per cent alcohol, dilute NH_4OH , dilute KOH and dilute HCl .

3. **Sublimation.**—Place a little tyrosin in a *dry* test-tube, heat gently and notice that the material does not sublime. How does this compare with the result of Experiment 3 under Leucin?

4. **Hoffman's Reaction.**—This is the name given to Millon's reaction when employed to detect tyrosin. Add about 3 c.c. of water and a few drops of Millon's reagent to a little tyrosin in a test-tube. Upon dissolving the tyrosin by heat the solution gradually darkens and may assume a dark red color. What group does this test show to be present in tyrosin?

5. **Piria's Test.**—Warm a little tyrosin on a watch glass on a boiling water-bath for 20 minutes with 3–5 drops of conc. H_2SO_4 . Tyrosin-sulphuric acid is formed in the process. Cool the solution and wash it into a small beaker with water. Now add CaCO_3 in substance slowly with stirring, until the reaction of the solution is no longer acid. Filter, concentrate the filtrate and add to it a few drops (avoid an excess) of very dilute neutral ferric chloride. A purple or violet color, due to the formation of the ferric salt of tyrosin-sulphuric acid, is produced. This is one of the most satisfactory tests for the identification of tyrosin.

6. **Mörner's Test.**—Add about 3 c.c. of Mörner's reagent¹ to a little tyrosin in a test-tube, and *gently* raise the temperature to the boiling-point. A green color results.

EXPERIMENTS ON LEUCIN.

Make the following tests upon the leucin crystals already prepared or upon some pure leucin furnished by the instructor.

1, 2 and 3. Repeat these experiments according to the directions given under Tyrosin (page 81).

¹ Mörner's reagent is prepared by thoroughly mixing 1 volume of formalin, 45 volumes of distilled water and 55 volumes of concentrated sulphuric acid.

CHAPTER V.

GASTRIC DIGESTION.

Gastric digestion takes place in the stomach and is promoted by the gastric juice, which is secreted by the glands of the stomach mucosa. These glands are of two kinds, *fundus* glands and *pyloric* glands which are situated, as their names imply, in the regions of the fundus and pylorus. The principal foods acted upon in gastric digestion are the proteids which are so changed by its processes as to become better prepared for further digestion in the intestine and for their final absorption.

From reliable experiments made upon lower animals it is evident that the gastric juice is secreted as the result of stimuli of two forms, *i. e.*, *psychical* stimuli and *chemical* stimuli. The psychical form of stimuli may be produced by the sight, thought or taste of food, and the chemical stimuli may be produced by certain substances such as water, the extractives of meat, etc., when coming in contact with the stomach mucosa. The volume of gastric juice secreted during any given period of digestion, varies with the quantity and kind of the food. These conclusions were deduced principally from a series of so-called *delusive feeding* experiments. A dog was prepared with two œsophageal openings and a gastric fistula. When thus prepared and fed foods of various kinds such as meat and bread, the material instead of passing to the stomach, would invariably find its way out of the animal's body at the upper œsophageal opening. Through the medium of the gastric fistula the course of the secretion of gastric juice could be carefully followed. It was found that when the dog ate meat, for example, there was a large secretion of gastric juice notwithstanding no portion of the food eaten had reached the stomach. Further experiments made through the medium of

a *cul-de-sac* formed from the stomach wall have given us many valuable conclusions, among others those regarding the influence of the chemical stimuli. The method followed was to feed the animal certain substances and note the secretion of gastric juice in the miniature stomach while the real process of digestion was taking place in the stomach proper.

Normal gastric juice is a thin, light colored fluid which is acid in reaction and has a specific gravity varying between 1.001 and 1.010. It contains only 2-3 per cent of solid matter which is made up principally of HCl, sodium chloride, potassium chloride, earthy phosphates, mucin and the enzymes pepsin, rennin and probably lipase; the HCl and the enzymes are of the greatest importance. The acidity of the gastric juice is due to *free* hydrochloric acid which is secreted by the parietal glands of the fundus and, in man, is generally present to the extent of 0.2-0.3 per cent. When the amount of HCl varies to any considerable degree from these values a condition of *hypoacidity* or *hyperacidity* is established. Hydrochloric acid has the power of combining with proteid substances taken in the food forming so-called *combined* hydrochloric acid. This combined acid is a less potent germicide than *free* HCl and has less power to destroy the amylolytic enzyme *ptyalin* of the saliva. This last fact explains to a degree the possibility of the continuance of salivary digestion in the stomach. The HCl of the gastric juice forms a medium in which the pepsin can most satisfactorily digest the proteid food, and at the same time it acts as an antiseptic or germicide which prevents putrefactive processes in the stomach. It also possesses the power of inverting cane sugar. When the HCl of the gastric juice is diminished in quantity (hypoacidity) or absent, as it may be in many cases of functional or organic disease, there is no check to the growth of micro-organisms in the stomach. There are however certain of the more resistant spores which even the normal acidity of the gastric juice will not destroy. A condition of hypoacidity may also give rise to fermentation with the formation of such bodies as lactic acid and butyric acid.

The most important of the enzymes of the gastric juice is the proteolytic enzyme *pepsin*. The pepsin does not originate as such in the gastric cells but is formed from its precursor the *zymogen* or mother-substance *pepsinogen* which is produced by the gastric cells. Upon coming in contact with the HCl of the secretion this pepsinogen is immediately transformed into pepsin. Pepsin is not active in alkaline or neutral solutions but requires at least a faint acidity before it can exert its power to dissolve and digest proteids. The percentage of HCl facilitating the most rapid peptic action varies with the character of the proteid acted upon, *e. g.*, 0.08 per cent to 0.1 per cent for the digestion of fibrin and 0.25 per cent for the digestion of coagulated egg-white. While HCl is the acid usually employed to promote artificial peptic proteolysis, other acids, organic and inorganic, will serve the same purpose. Acidity of the liquid is necessary to promote the activity of the pepsin, but the acidity need not necessarily be confined to hydrochloric acid.

In common with many other enzymes pepsin acts best at about 38°-40° C. and its digestive power decreases as the temperature is lowered, the enzyme being only slightly active at 0° C. Its power is only temporarily inhibited by the application of such low temperatures, however, and the enzyme regains its full proteolytic power upon raising the temperature to 40° C. As the temperature of a digestive mixture is raised above 40° C. the pepsin gradually loses its activity until at about 80°-100° C. its proteolytic power is permanently destroyed.

The products of peptic proteolysis are acid albuminate, proteoses (albumoses) and peptones. Only a comparatively small amount of the proteid ingested is transformed into peptones, the proteose stage being, for the most part, the final stage in peptic proteolysis. The relative amounts of proteoses and peptones formed depends to a great extent upon the character of the proteid undergoing digestion, *e. g.*, a greater proportion of proteoses results from the digestion of fibrin than from the

digestion of coagulated egg-white. Peptic proteolysis differs from tryptic proteolysis (see page 107) in that the former yields larger amounts of proteoses, smaller amounts of peptones and no considerable quantity of crystalline bodies as end-products in the brief period during which proteids are ordinarily subjected to gastric digestion. Prolonged hydrolysis with gastric juice may however, yield considerable quantities of the non-proteid end-products.

Rennin, the second enzyme of the gastric juice, is what is known as a *milk curdling* or *proteid coagulating* enzyme. Rennin acts upon the caseinogen of the milk, splitting it into a *protease-like* body and *soluble casein*. This soluble body, in the presence of calcium salts, combines with calcium forming *calcium casein* or true *casein* which is insoluble and precipitates. There is some uncertainty regarding the reaction, to litmus, in which rennin shows the greatest activity. It is, however, usually said to be most active in the presence of a slight acid reaction, as would naturally be expected. It is especially abundant in the gastric mucosa of the calf, and is used to curdle the milk used in cheese making. Rennin is always present normally in the gastric juice but in certain pathological conditions such as atrophy of the mucosa, chronic catarrh of the stomach or in carcinoma it may be absent.

Lipase is a fat-splitting enzyme (see page 97).

PREPARATION OF AN ARTIFICIAL GASTRIC JUICE.

Dissect the mucous membrane of a pig's stomach from the muscular portion, and discard the latter. Divide the mucous membrane into two parts ($\frac{4}{5}$ and $\frac{1}{5}$). Cut up the larger portion, place it in a large-sized beaker with 0.4 per cent HCl and keep at 38° – 40° C. for at least 24 hours. Filter off the residue, consisting principally of nuclein and anti-albumid, and use the filtrate as an artificial gastric juice. This filtrate contains pepsin, rennin and the products of the digestion of the stomach tissue, *i. e.*, acid albuminate, proteoses and peptones.

PREPARATION OF A GLYCERIN EXTRACT OF PIG'S STOMACH.

Take the one-fifth portion of the mucous membrane of the pig's stomach not used in the preparation of the artificial gastric juice, cut it up very finely, place it in a small-sized beaker and cover the membrane with glycerin. Stir frequently and allow to stand at room temperature for at least 24 hours. The glycerin will extract the *pepsinogen*. Separate, with a pipette or by other means, the glycerin from the pieces of mucous membrane and use the glycerin extract as required in the later experiments.

PRODUCTS OF GASTRIC DIGESTION.

Into the artificial gastric juice, prepared as above described, place the proteid material (fibrin, coagulated egg-white, or lean beef) provided for you by the instructor, add 0.4 per cent HCl as suggested by the instructor and keep the digestion mixture at 40° C. for 2 to 3 days. Stir frequently and keep *free* hydrochloric acid present in the solution (for tests for free HCl see below).

The original proteid has been digested and the solution now contains the products of peptic proteolysis, *i. e.*, acid albuminate, proteoses and peptones. The insoluble residue may include nuclein and anti-albumid. Filter the digestive mixture and after testing for *free* HCl neutralize the filtrate with KOH solution. If any of the acid albuminate is still untransformed into proteoses it will precipitate upon neutralization. If any precipitate forms heat the mixture to boiling, and filter. If no precipitate forms proceed without filtering.

We now have a solution containing a mixture of proteoses and peptones. Separate and identify these bodies according to the directions given on pages 59 and 60.

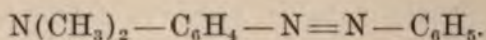
Tests for Free and Combined HCl.

These tests are made with a class of reagents known as *indicators*, so-called because they serve to indicate the nature of the reaction of a solution. These indicators are weak acids

or bases and are but slightly dissociable. The dissociation, with the formation of the colored ion, forms the basis for the color reaction.

Examine each of the following solutions by means of the tests given below and report the results in a form similar to the chart given on page 90: (1) 0.2 per cent *free* HCl. (2) 0.05 per cent *free* HCl. (3) 0.01 per cent *free* HCl. (4) 0.05 per cent *combined* HCl. (5) 1 per cent lactic acid. (6) Equal volumes of 0.2 per cent *free* HCl and 1 per cent lactic acid. (7) 1 per cent potassium hydroxide.

1. Di-methyl-amino-azobenzene (or Töpfer's Reagent),¹



Place 1-2 drops of the reagent in the solution to be tested. Free mineral acid (HCl) is indicated by the production of a pinkish-red color. If free acid is absent a yellow color ordinarily results.

2. Günzberg's Reagent.²—Place 1-2 drops of the reagent in a small porcelain evaporating dish and *carefully* evaporate to dryness over a *low* flame. Insert a glass stirring rod into the mixture to be tested and draw the moist end of the rod through the dried reagent. Warm again gently and note the production of a purplish-red color in the presence of *free* HCl.

3. Boas' Reagent.³—Perform this test in the same manner as 2 above. Free hydrochloric acid is indicated by the production of a rose-red color which becomes less pronounced on cooling.

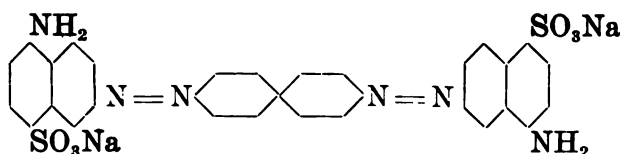
4. Congo Red,⁴

¹ To prepare Töpfer's reagent dissolve 0.5 gram of di-methyl-amino-azobenzene in 100 c.c. of 95 per cent alcohol.

² Günzberg's reagent is prepared by dissolving 2 grams of phloroglucin and 1 gram of vanillin in 100 c.c. of 95 per cent alcohol.

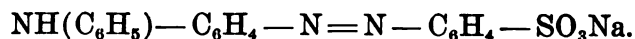
³ Boas' reagent is prepared by dissolving 5 grams of resorcin and 3 grams of saccharose in 100 c.c. of 95 per cent alcohol.

⁴ This indicator is prepared by dissolving 0.5 gram of congo red in 90 c.c. of water and adding 10 c.c. of 95 per cent alcohol.



Conduct this test according to the directions given under 1 or 2 page 88. A blue color indicates free HCl, a violet color indicates an organic acid and a brown color indicates combined HCl. Congo red paper made by immersing ordinary filter paper in the indicator and subsequently drying, may be used in this test.

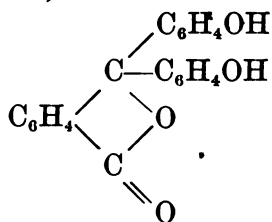
5. Tropæolin OO,¹



Place 2 drops of the solution to be tested and 1 drop of the indicator in an evaporating dish and evaporate to dryness over a low flame. The formation of a reddish-violet color indicates *free* hydrochloric acid.

This test may also be conducted in the same manner as 2 page 88.

✓ 6. Phenolphthalein,²



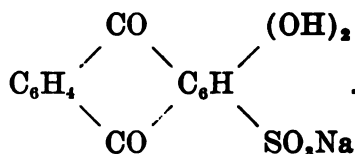
Add the indicator directly to the solution, or apply the test according to the directions given under 2 on page 88. This indicator serves to denote the *total acidity* since it is acted upon by free mineral acids, combined acids, organic acids and acid salts. A red color indicates the presence of an alkali and

¹ Prepared by dissolving 0.05 gram of tropæolin OO in 100 c.c. of 50 per cent alcohol.

² This indicator is prepared by dissolving 1 gram of phenolphthalein in 100 c.c. of 95 per cent alcohol.

the indicator is colorless in the presence of a neutral or acid reaction. This indicator is unsatisfactory in the presence of ammonia.

7. Sodium Alizarin Sulphonate,¹



This indicator may be used directly in the solution to be tested, or the test may be applied as 2, page 88. It serves to indicate all acid reactions except those due to *combined* acids. A reddish-violet color indicates an alkaline reaction, while a yellow color indicates an acid reaction due to a free mineral acid, an organic acid or an acid salt.

Report the results of your tests tabulated in the form given below:

Name of Indicator.	Solutions Examined.					
	0.2 % HCl.	0.05 % HCl.	0.01 % HCl.	0.05 % Combined HCl.	1 % Lactic Acid.	Equal Vols. 0.2 % HCl and 1 % Lactic Acid.
Töpfer's Reagent.						
Günzberg's Reagent.						
Boas' Reagent.						
Congo Red.						
Tropeolin OO.						
Phenolphthalein.						
Alizarin.						

¹ Prepare this indicator by dissolving 1 gram of sodium alizarin sulphonate in 100 c.c. of water.

GENERAL EXPERIMENTS ON GASTRIC DIGESTION.

1. **Conditions Essential for the Action of Pepsin.**—Prepare four test-tubes as follows:

- (a) Five c.c. of pepsin solution.
- (b) Five c.c. of 0.4 per cent HCl.
- (c) Five c.c. of pepsin-hydrochloric acid solution.
- (d) Two or three c.c. of pepsin solution and 2-3 c.c. of 0.5 per cent sodium carbonate solution.

Introduce into each tube a small piece of fibrin and place them on the water-bath at 40° C. for one-half hour, carefully noting any changes which occur.¹ Now combine the contents of tubes (a) and (b) and see if any further change occurs after standing at 40° C. for 15-20 minutes. Explain the results obtained from these five experiments.

2. **Influence of Different Temperatures.**—In each of four test-tubes place 5 c.c. of pepsin-hydrochloric acid solution. Immerse one tube in cold water from the faucet, keep a second tube at room temperature and place a third on the water-bath at 40° C. Boil the contents of the fourth tube for a few moments then cool and also keep it at 40° C. Into each tube introduce a small piece of fibrin and note the progress of digestion. In which tube does the most rapid digestion occur? Explain this.

3. **The Most Favorable Acidity.**—Prepare three tubes as follows:

- (a) Five c.c. of 0.2 per cent pepsin-hydrochloric acid solution.

¹ Digestion of fibrin in a pepsin-hydrochloric acid solution is indicated first by a *swelling* of the proteid due to the action of the acid, and later by a *disintegration* and *dissolving* of the fibrin due to the action of the pepsin-hydrochloric acid. If uncertain at any time whether digestion has taken place, the solution under examination may be filtered and the biuret test applied to the filtrate. A positive reaction will signify the presence of acid albuminate, proteoses (albumoses) or peptones, the presence of any one of which would indicate that digestion has taken place.

(b) Two or three c.c. of 0.2 per cent HCl + 1 c.c. of concentrated HCl + 5 c.c. of pepsin solution.

(c) One c.c. of 0.2 per cent pepsin-hydrochloric acid solution + 5 c.c. of water.

Introduce a small piece of fibrin into each tube, keep them at 40° C., and note the progress of digestion. In which degree of acidity does the fibrin digest the most rapidly?

4. **Differentiation Between Pepsin and Pepsinogen.**—Prepare five tubes as follows:

(a) Few drops of glycerin extract of pepsinogen + 2-3 c.c. of water.

(b) Few drops of glycerin extract of pepsinogen + 5 c.c. of 0.2 per cent HCl.

(c) Few drops of glycerin extract of pepsinogen + 5 c.c. of 0.5 per cent Na_2CO_3 .

(d) Two or three c.c. of pepsin solution + 2-3 c.c. of 1 per cent Na_2CO_3 .

(e) Few drops of glycerin extract of pepsinogen + 5 c.c. of 1 per cent Na_2CO_3 .

Add a small piece of fibrin to the contents of each tube, keep the five tubes at 40° C. for one-half hour and observe any changes which may have occurred. To (a) add an equal volume of 0.4 per cent HCl, neutralize (c), (d) and (e) with HCl and add an equal volume of 0.4 per cent HCl. Place these tubes at 40° C. again and note any further changes which may occur. What contrast do we find in the results from the three last tubes? Why is this so?

5. **Comparative Digestive Power of Pepsin with Different Acids.**—Prepare a series of tubes each containing one of the following acids: 0.5 per cent acetic, lactic, oxalic and butyric, and 0.2 per cent hydrochloric, sulphuric, nitric and *combined* hydrochloric. To each acid add a few drops of the glycerin extract of pig's stomach and a small piece of fibrin. Shake well, place at 40° C. and note the progress of digestion. In which tubes does the most rapid digestion occur?

6. **Influence of Metallic Salts, etc.**—Prepare a series of

tubes and into each tube introduce 4 c.c. of pepsin-hydrochloric acid solution and $\frac{1}{2}$ c.c. of one of the chemicals listed in Experiment 18 under Salivary Digestion, page 40. Introduce a small piece of fibrin into each of the tubes and keep them at 40° C. for one-half hour. Note the variations in the progress of digestion. Where has the least rapid digestion occurred?

7. Testing the Motor and Functional Activities of the Stomach.—This test is performed the same as Experiment 19 under Salivary Digestion, page 40. If the experiment was carried out under salivary digestion it will not be necessary to repeat it here.

8. Influence of Bile.—Prepare five tubes as follows:

(a) Five c.c. of pepsin-hydrochloric acid solution + $\frac{1}{2}$ –1 c.c. of bile.

(b) Five c.c. of pepsin-hydrochloric acid solution + 1–2 c.c. of bile.

(c) Five c.c. of pepsin-hydrochloric acid solution + 2–3 c.c. of bile.

(d) Five c.c. of pepsin-hydrochloric acid solution + 5 c.c. of bile.

(e) Five c.c. of pepsin-hydrochloric acid solution.

Introduce into each tube a small piece of fibrin. Keep the tubes at 40° C. and note the progress of digestion. Does the bile exert any appreciable influence? How?

9. Influence of Rennin on Milk.—Prepare a series of five tubes as follows:

(a) Five c.c. of fresh milk + 0.2 per cent HCl (add slowly until precipitate forms).

(b) Five c.c. of fresh milk + 5 drops of rennin solution.

(c) Five c.c. of fresh milk + 10 drops of 0.5 per cent Na_2CO_3 solution.

(d) Five c.c. of fresh milk + 10 drops of 5 per cent ammonium oxalate solution.

(e) Five c.c. of fresh milk + 5 drops of 0.2 per cent HCl.

Now to each of the tubes (c), (d) and (e) add 5 drops of

rennin solution. Place the whole series of five tubes at 40° C. and after 10–15 minutes note what is occurring in the different tubes. Give a reason for each particular result.

10. Tests for Lactic Acid. (a) *Uffelmann's Reaction*.—To a small quantity of Uffelmann's reagent¹ in a test-tube add a few drops of a lactic acid solution. The amethyst-blue color of the reagent is displaced by a straw yellow. Other organic acids give a similar reaction. Mineral acids such as HCl discharge the blue coloration leaving a colorless solution.

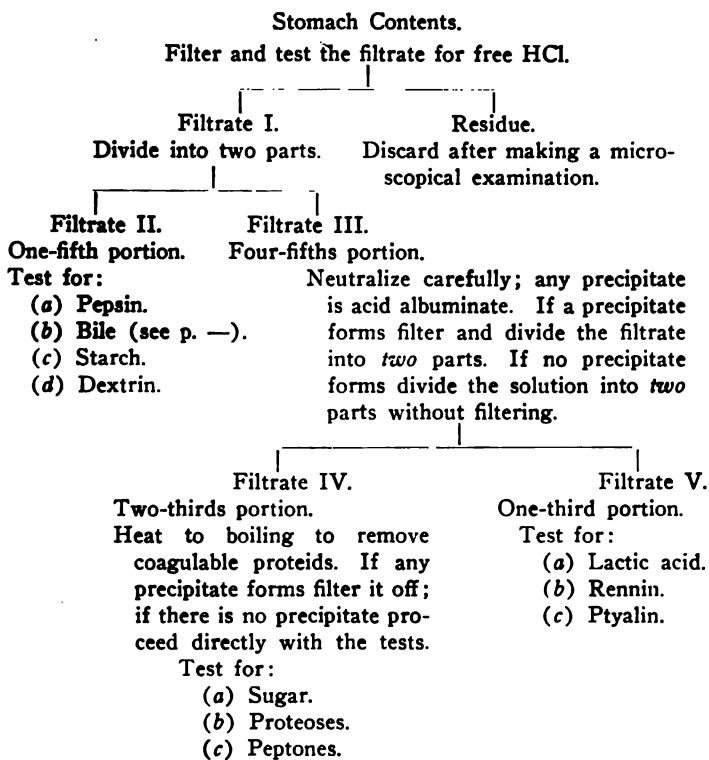
(b) *Ferric Chloride Test*.—Place 10 c.c. of *very dilute* ferric chloride in each of five tubes. To the first add 2 c.c. of 0.2 per cent HCl, to the second 2 c.c. of 10 per cent alcohol, to the third 2 c.c. of 2 per cent saccharose, to the fourth 2 c.c. of lactic acid and to the fifth 2 c.c. of peptone solution.

It is evident from the results obtained that neither of the tests given above is satisfactory for the detection of lactic acid in the presence of other substances such as we find in the gastric contents.

A satisfactory deduction regarding the presence of lactic acid can only be made after extracting the gastric contents with ether, evaporating the ether extract to dryness and dissolving the residue in water. This residue will not contain any of the contaminations which interfered with the simple tests as tried above, and therefore if either of the tests is now tried on the dissolved residue of the ether extract we may form an accurate conclusion regarding the presence of lactic acid.

11. Qualitative Analysis of Stomach Contents.—Take 100 c.c. of stomach contents and analyze it according to the following scheme:

¹ Uffelmann's reagent is prepared by adding ferric chloride solution to a 1 per cent solution of carbolic acid until an amethyst-blue color is obtained.



CHAPTER VI.

FATS.

Fats occur very widely distributed in the plant and animal kingdoms, and constitute the third general class of food stuffs. In plant organisms they are to be found in the seeds, roots and fruit, while each individual tissue and organ of an animal organism contains more or less of the substance. In the animal organism fats are especially abundant in the bone marrow and adipose tissue. They contain the same elements as the carbohydrates, *i. e.*, carbon, hydrogen and oxygen, but the oxygen is present in smaller percentage than in the carbohydrates and the hydrogen and oxygen are not present in the proportion to form water. According to the observa-

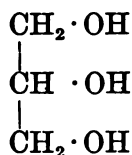
FIG. 35.



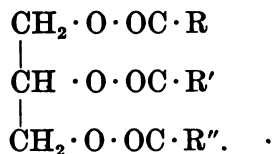
BEEF FAT. (*Long.*)

tions of Benedict and Osterberg human fat contains 76.08 per cent of carbon and 11.78 per cent of hydrogen. They found the heat of combustion of human fat to be 9.523 calories per gram.

Chemically considered the fats are esters¹ of the tri-atomic alcohol, glycerin, and the mono-basic fatty acids. The H of each of the OH groups of glycerin is replaced by a fatty acid radical (see page 65). For instance

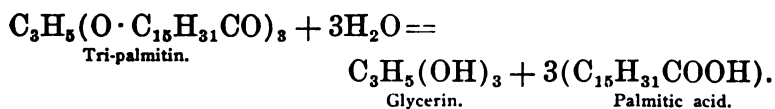


is the formula for glycerin and by replacing the hydrogen of the hydroxyls by hydrocarbon radicals R, R' and R'' we obtain, as the typical formula for an ordinary neutral fat,



The positions occupied by R, R' and R'' in the above formula may be filled by three radicals of the same fatty acid or by the radicals of three different fatty acids.

By hydrolysis of a neutral fat, *i. e.*, by the addition to the molecule of those elements which are eliminated in the formation of the fat from glycerin and fatty acid, it may be resolved into its component parts, *i. e.*, glycerin and fatty acid. In the case of tri-palmitin the following would be the reaction:



This process is called *saponification* and may be produced by boiling with alkalis; by the action of steam under pressure; by long-continued contact with air and light; by the action of certain bacteria and by fat-splitting enzymes or lipases, *e. g.*,

¹ An ester is an ethereal salt consisting of an organic radical united with the residue of an inorganic or organic acid.

steapsin (see page 109). The cells forming the walls of the intestines evidently possess the peculiar property of synthesizing the glycerin and fatty acid thus formed so that after absorption these bodies appear in the blood not in their individual forms but as neutral fats. This synthesis is similar to that enacted in the absorption of proteid material where the peptones are synthesized into albumin in the act of absorption.

The principal animal fats with which we have to deal are *stearin*, *palmitin*, *olein* and *butyrin*. Such less important forms as *laurin* and *myristin* may occur abundantly in plant organisms. The generally accepted system of nomenclature for these fats is to apply the prefix "tri" in each case (*e. g.*, *tri-palmitin*) since three fatty acid radicals are contained in the neutral fat molecule.

Fats occur ordinarily as mixtures of several individual fats. For example, the fat found in animal tissues is a mixture of *tri-olein*, *tri-palmitin* and *tri-stearin*, the percentage of any one of these fats present depending upon the particular species of animal from whose tissue the fat was derived. Thus the ordinary mutton fat contains more *tri-stearin* and less *tri-olein* than the pork fat. The crystalline forms of some of the more common fats are reproduced in Figs. 35, 36 and 37 on pages 96, 99 and 101.

Pure neutral fats are odorless, tasteless and generally colorless. They are insoluble in the ordinary proteid solvents such as water, sodium chloride and dilute acids and alkalis but are very readily soluble in ether, benzene, chloroform and *boiling* alcohol. Each individual fat possesses a specific melting- or boiling-point (according to whether the body is solid or fluid in character) and this property of melting or boiling at a definite temperature may be used as a means of differentiation in the same way as the coagulation temperature (see page 60) is used for the differentiation of coagulable proteids. When shaken with water, or a solution of albumin, soap or gum

arabic, the fats are finely divided and assume a condition known as an *emulsion*. The emulsion with water is transitory, while the emulsions with soap or albumin solution are permanent.

The fat ingested continues essentially unaltered until it reaches the intestines where it is acted upon by *steapsin* the fat-splitting enzyme of the pancreatic juice (see page 109), and glycerin and fatty acid are formed from a large portion of the fat. Part of the fatty acid thus formed is dissolved in the bile and absorbed while the remainder unites with the alkalis of the pancreatic juice and forms soluble soaps. These soaps may further act to produce an emulsion of the remaining fat and thus aid in its absorption. That bile is of assistance in the absorption of fat is indicated by the increase of fat in the feces when for any reason bile does not pass into the intestines.

The fat distributed throughout the animal body is formed partly from the ingested fat and partly from carbohydrates

FIG. 36.



MUTTON FAT. (Long.)

and the "carbon moiety" of proteid material. The formation of *adipocere* and the occurrence of *fatty degeneration* are sometimes given as proofs of the formation of fat from pro-

teid. This is questioned by many investigators. Rather more satisfactory and direct proof of the formation of fat from proteid material has been obtained by experimentation upon *fly-maggots*. The normal content of fat in a number of maggots was determined and later the fat content of others which had developed in blood (84 per cent of the solid matter of blood plasma is proteid material) was determined. The fat content was found to have increased 700 to 1100 per cent as a result of the diet of blood proteids. Some investigators are not inclined to accept these data as conclusive.

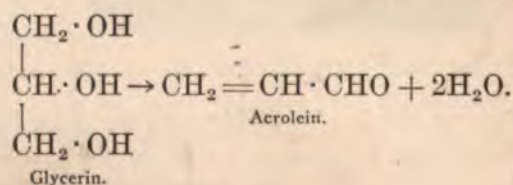
EXPERIMENTS ON FATS.

1. **Solubility.**—Test the solubility of olive oil in each of the ordinary solvents (see page 4) and in cold alcohol, hot alcohol, chloroform and ether.

2. **Formation of a Transparent Spot on Paper.**—Place a drop of olive oil upon a piece of ordinary writing paper. Note the transparent appearance of the paper at the point of contact with the fat.

3. **Reaction.**—Try the reaction of *fresh* olive oil to litmus. Repeat the test with *rancid* olive oil. What is the reaction of a fresh fat and how does this reaction change upon allowing the fat to stand for some time?

4. **Formation of Acrolein.**—To a little olive oil in a mortar add some potassium bisulphate, KHSO_4 , and rub up thoroughly. Transfer to a *dry* test-tube and cautiously heat. Note the irritating odor of *acrolein*. The glycerin of the fat has been dehydrolyzed and acrylic aldehyde or acrolein has been produced. This is the reaction which takes place:



5. **Emulsification.**—(a) Shake up a drop of *neutral*¹ olive oil with a little water in a test-tube. The fat becomes finely divided, forming an emulsion. This is not a permanent emulsion since the fat separates and rises to the top upon standing.

(b) To 5 c.c. of water in a test-tube add 2 or 3 drops of 0.5 per cent Na_2CO_3 . Introduce into this faintly alkaline solution a drop of *neutral* olive oil and shake. The emulsion while not permanent is not so transitory as in the case of water free from sodium carbonate.

(c) Repeat (b) using *rancid* olive oil. What sort of an emulsion do you get and why?

(d) Shake a drop of *neutral* olive oil with a dilute albumin solution. What is the nature of this emulsion? Examine it under the microscope.

6. **Fat Crystals.**—Dissolve a small piece of lard in ether in a test-tube, add an equal volume of alcohol and allow the alcohol-ether mixture to evaporate spontaneously. Examine

FIG. 37.



PORK FAT.

¹ Neutral olive oil may be prepared by shaking ordinary olive oil with a 10 per cent solution of sodium carbonate. This mixture should then be extracted with ether and the ether removed by evaporation. The residue is *neutral* olive oil.

the crystals under the microscope and compare them with those reproduced in Figs. 35, 36 and 37 on pp. 96, 99 and 101.

7. Saponification of Bayberry Tallow.—Fill a large casserole two-thirds full of water rendered strongly alkaline with *solid* KOH (a stick one inch in length). Add about 10 grams of bayberry tallow and boil, keeping the volume constant by adding water as needed. When saponification is complete¹ add concentrated HCl slowly until no further precipitate is produced. Cool the solution and the precipitate of free fatty acid will rise to the surface and form a cake. In this instance the fatty acid is principally *palmitic* acid. Remove the cake, break it into small pieces, wash it with water by decanta-

FIG. 38.



PALMITIC ACID.

tion and transfer to a small beaker by means of 95 per cent alcohol. Heat on a water-bath until the palmitic acid is dissolved, then filter through a dry filter paper and allow the filtrate to cool slowly in order to obtain satisfactory crystals. Write the reactions which have taken place in this experiment.

When the palmitic acid has completely crystallized filter

¹ Place 2 or 3 drops in a test-tube full of water. If saponification is complete the products will remain in solution.

off the alcohol, dry the crystals between filter papers and try the tests given below.

8. **Palmitic Acid.**—(a) Examine the crystals under the microscope and compare them with those shown in Fig. 38, opposite.

(b) *Solubility.*—Try the solubility of palmitic acid in the same solvents as used on fats (see p. 100).

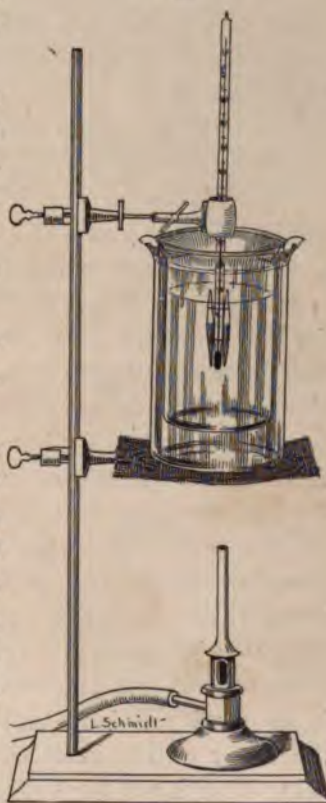
(c) *Melting-Point.*—Determine the melting-point of palmitic acid by one of the methods given on pages 104 and 105.

(d) *Formation of Transparent Spot on Paper.*—Melt a little of the fatty acid and allow a drop to fall upon a piece of ordinary writing paper. How does this compare with the action of a fat under similar circumstances?

(e) *Acrolein Test.*—Apply the test as given under 4, page 100. Explain the result.

9. **Saponification of Lard.**—To 25 grams of lard in a flask add 75 c.c. of alcoholic-potash solution and warm upon a water-bath until saponification is complete. (This point is indicated by the complete solubility of a drop of the solution when allowed to fall into a little water.) Now transfer the solution from the flask to an evaporating dish containing about 100 c.c. of water and heat on a water-bath until all the alcohol has been driven off. Precipitate the fatty acid with HCl and cool the solution. Remove the fatty acid which rises to the surface,

FIG. 39.



MELTING-POINT APPARATUS.

neutralize the solution with Na_2CO_3 and evaporate to dryness. Extract the residue with alcohol, remove the alcohol by evaporation upon a water-bath and on the residue of glycerin thus obtained make the tests as given below.

10. **Glycerin.** (a) *Taste*.—What is the taste of glycerin?

(b) *Solubility*.—Try the solubility of glycerin in water, alcohol and ether.

(c) *Acrolein Test*.—Repeat the test as given under 4, p. 100.

(d) *Borax Fusion Test*.—Fuse a little glycerin on a platinum wire with some powdered borax and note the characteristic green flame. This color is due to the glycerin ester of boric acid.

(e) *Fehling's Test*.—How does this result compare with the results on the sugars?

(f) *Solution of $\text{Cu}(\text{OH})_2$* .—Form a little $\text{Cu}(\text{OH})_2$ by mixing CuSO_4 and KOH . Add a little glycerin to this suspended precipitate and note what occurs.

11. **Melting-Point of Fat.** *First Method*.—Insert one of the melting-point tubes, furnished by the instructor, into the liquid fat and draw up the fat until the bulb of the tube is about one-half full of the material. Then fuse one end of the tube in the flame of a bunsen burner and fasten the tube to a thermometer by means of a rubber band in such a manner that the bottom of the fat column is on a level with the bulb of the thermometer (Fig. 39, p. 103). Fill a beaker of medium size about two-thirds full of water and place it within a second larger beaker which also contains water, the two vessels being separated by pieces of cork. Immerse the bulb of the thermometer and the attached tube in such a way that the bulb is about midway between the upper and the lower surfaces of the water of the inner beaker. The upper end of the tube being open it must extend above the surface of the surrounding water. Apply gentle heat, stir the water, and note the temperature at which the fat first begins to melt. This point is indicated by the initial transparency. For ordinary fats, raise the temperature very cautiously from 30°C . To deter-

mine the *congealing-point* remove the flame and note the temperature at which the fat begins to solidify. Record the melting- and congealing-points of the various fats submitted by the instructor.

Second Method.—Fill a small evaporating dish about one-half full of mercury and place it on a water-bath. Put a small drop of the fat under examination on an ordinary cover glass and place this upon the surface of the mercury. Raise the temperature of the water-bath slowly and by means of a thermometer whose bulb is immersed in the mercury note the melting-point of the fat. Determine the congealing-point by removing the flame and leaving the fat drop and cover glass in position upon the mercury. How do the melting-points as determined by this method compare with those as determined by the first method? Which method is the more accurate, and why?

CHAPTER VII.

PANCREATIC DIGESTION.

As soon as the food mixture leaves the stomach it comes into intimate contact with the bile and the pancreatic juice. Since these fluids are alkaline in reaction there can obviously be no further peptic activity after they have become intimately mixed with the chyme and have neutralized the acidity previously imparted to it by the hydrochloric acid of the gastric juice. The pancreatic juice reaches the intestine through the duct of Wirsung which opens into the intestine near the pylorus.

Normally the secretion of pancreatic juice is brought about by the stimulation produced by the acid chyme as it enters the duodenum. This secretion is probably not due to a nervous reflex, but is dependent upon the presence, in the epithelial cells of the duodenum, of a body known as *prosecretin*. This body is changed into *secretin* through the hydrolytic action of the acid present in the chyme. The secretin is then absorbed by the blood, passes to the pancreas and stimulates the pancreatic cells, causing a flow of pancreatic juice. The quantity of juice secreted under these conditions is proportional to the amount of secretin present. The activity of secretin solutions is not diminished by boiling, hence the body does not react like an enzyme. Further study of the body may show it to be a definite chemical individual of relatively low molecular weight. It has not been possible thus far to obtain secretin from any tissues except the mucus membrane of the duodenum and jejunum.

The juice as obtained from a permanent fistula differs greatly in its properties from the juice as obtained from a temporary fistula, and neither form of fluid possesses the properties of the normal fluid. Pancreatic juice collected from

a natural fistula has been found to be a colorless, clear, strongly alkaline fluid which foams readily. It is further characterized by containing albumin and globulin and by the absence of proteoses and peptone. The average daily secretion of pancreatic juice is 650 c.c. and its specific gravity is 1.008. The fluid contains 1.3 per cent of solid matter and the freezing-point is -0.47° C. The normal pancreatic secretion contains at least four distinct enzymes. They are *trypsin*, a proteolytic enzyme; *amyllopsin*, an amylolytic enzyme; *steapsin*, a fat-splitting enzyme; and *pancreatic rennin*, a milk-coagulating enzyme.

The most important of the four enzymes of the pancreatic juice is the proteolytic enzyme *trypsin*. This enzyme resembles pepsin in so far as each has the power of breaking down proteid material, but the trypsin has much greater digestive power and is able to cause a more complete decomposition of the complex proteid molecule. In the process of normal digestion the proteid constituents of the diet are for the most part transformed into proteoses (albumoses) before coming in contact with the enzyme trypsin. This is not absolutely essential however, since trypsin possesses digestive activity sufficient to transform unaltered native proteids and to produce from their complex molecules comparatively simple fragments. Among the products of tryptic digestion are *alkali albuminate*, *proteoses* (albumoses), *peptone*, *leucin*, *tyrosin*, *aspartic acid*, *glutamic acid*, *lysin*, *histidin*, *arginin*, *tryptophan* and *ammonia*. (The crystalline forms of many of these products are reproduced in Chapter IV.) Trypsin does not occur preformed in the gland, but exists there as a zymogen called *trypsinogen* which bears the same relation to trypsin that pepsinogen does to pepsin. Trypsin has never been obtained in a pure form and therefore very little can be stated definitely as to its nature. The enzyme is the most active in alkaline solution but is also active in neutral or slightly acid solutions. Trypsin is destroyed by mineral acids and may also be destroyed by comparatively weak alkali (2 per cent sodium car-

bonate) if left in contact for a sufficiently long time. Trypsinogen, on the other hand, is more resistant to the action of alkalis.

The pancreatic juice which is collected by means of a fistula possesses practically no power to digest proteid matter. A body called *enterokinase* occurs in the intestinal juice and has the power of converting trypsinogen into trypsin. This process is known as the "activation" of trypsinogen and through it a juice which is incapable of digesting proteid may be made active. Enterokinase is not always present in the intestinal juice since it is secreted only after the pancreatic juice reaches the intestine. It resembles the enzymes in that its activity is destroyed by heat, but differs materially from this class of bodies in that a certain quantity of intestinal juice is capable of activating only a definite quantity of trypsinogen. Enterokinase has been detected in the higher animals, and a kinase possessing similar properties has been shown to be present in bacteria, fungi, impure fibrin, lymph glands and snake-venom. The activation of trypsinogen into trypsin may be brought about in the gland as well as in the intestine of the living organism (Mendel and Rettger). The manner of the activation in the gland and the nature of the body causing it are unknown at present.

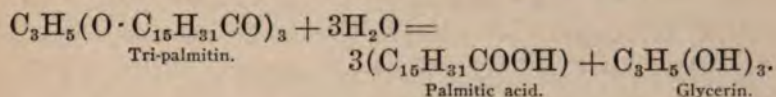
Amylopsin, the second of the pancreatic enzymes, is an amylolytic enzyme which possesses somewhat greater digestive power than the ptyalin of the saliva. As its name implies, its activity is confined to the starches, and the products of its amylolytic action are dextrins and sugars. The sugars are principally iso-maltose and maltose and these by the further action of an inverting enzyme are partly transformed into dextrose.

It is probable that the saliva as a digestive fluid is not absolutely essential. The ptyalin is destroyed by the hydrochloric acid of the gastric juice and is therefore inactive when the chyme reaches the intestine. Should undigested starch be present at this point however, it would be quickly transformed

by the active amylopsin. This enzyme is not present in the pancreatic juice of infants during the first few weeks of life, thus showing very clearly that a starchy diet is not normal for this period.

It has been claimed that amylopsin has a slight digestive action upon *unboiled* starch.

The third enzyme of the pancreatic juice is called *steapsin* and is a fat-splitting enzyme. It has the power of splitting the neutral fats of the food by hydrolysis, into fatty acid and glycerin. A typical reaction would be as follows:



Recent researches make it probable that fats undergo saponification to a very large extent prior to their absorption. The fatty acids formed, in part unite with the alkalis of the pancreatic juice and intestinal secretion to form soluble soaps; in part they are doubtless absorbed dissolved in the bile. Some observers believe that the fats may also be absorbed in emulsion—a condition promoted by the presence of the soluble soaps. After absorption the fatty acids are re-synthesized to form neutral fats with glycerin.

Steapsin is very unstable and is easily rendered inert by the action of acid. For this reason it is not possible to prepare an extract having a satisfactory fat-splitting power from a pancreas which has been removed from the organism for a sufficiently long time to have become acid in reaction.

The fourth enzyme of the pancreatic juice is called *pancreatic rennin*. It is a milk-coagulating enzyme whose action is very similar to that of the enzyme *rennin* found in the gastric juice. It is supposed to show its greatest activity at a temperature varying from 50° to 60° C.

PREPARATION OF AN ARTIFICIAL PANCREATIC JUICE.

After removing the fat from the pancreas of a pig or sheep, finely divide the organ by means of scissors and grind it in a mortar. If convenient, the use of an ordinary meat chopper is a very satisfactory means of preparing the pancreas.

When finely divided as above the pancreas should be placed in a 500 c.c. flask, about 150 c.c. of 30 per cent alcohol added and the flask and contents shaken frequently for twenty-four hours. (What is the reaction of this alcoholic extract at the end of this period, and why?) Strain the alcoholic extract through cheese cloth, filter, nearly neutralize with KOH solution and then exactly neutralize it with 0.5 per cent Na_2CO_3 .

PRODUCTS OF PANCREATIC DIGESTION.

Take about 200 grams of lean beef which has been freed from fat and finely ground and place it in a large-sized beaker. Nearly fill the beaker with pancreatic extract prepared as above, add 5 c.c. of an alcoholic solution of thymol to prevent putrefaction, and place the beaker in an incubator at 40°C . Stir the contents of the beaker frequently and add more thymol if it becomes necessary. Allow digestion to proceed for from 2 to 5 days and then separate the products formed as follows: Strain off the undissolved residue through cheese cloth, nearly neutralize the solution with dilute hydrochloric acid and then exactly neutralize it with 0.2 per cent hydrochloric acid. A precipitate at this point would indicate *alkali albuminate*. Filter off any precipitate and divide the filtrate into two parts, a one-fourth and a three-fourth portion.

Transfer the one-fourth portion to an evaporating dish and make the separation of *proteoses* and *peptones* as well as the final tests upon these bodies according to the directions given on page 59.

Place about 5 c.c. of the three-fourth portion in a test-tube and add about 1 c.c. of bromine water. A violet coloration

indicates the presence of *tryptophan* (see page 77). Concentrate¹ the remainder of the three-fourth portion to a thin syrup and make the separation of *leucin* and *tyrosin* according to the directions given on page 81.

GENERAL EXPERIMENTS ON PANCREATIC DIGESTION.

EXPERIMENTS ON TRYPSIN.

1. The Most Favorable Reaction for Tryptic Digestion.

—Prepare seven tubes as follows:

(a) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of water.

(b) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 1 per cent Na_2CO_3 .

(c) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 0.5 per cent Na_2CO_3 .

(d) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 0.2 per cent HCl .

(e) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 0.2 per cent *combined* HCl .

(f) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 0.4 per cent boric acid.

(g) 2-3 c.c. of *neutral* pancreatic extract + 2-3 c.c. of 0.4 per cent acetic acid.

Add a small piece of fibrin to the contents of each tube and keep them at 40° C. noting the progress of digestion. In which tube do we find the most satisfactory digestion, and why? How do the indications of the digestion of fibrin by trypsin differ from the indications of the digestion of fibrin by pepsin?

2. The Most Favorable Temperature.—(For this and the following series of experiments under tryptic digestion use the *neutral* extract plus an *equal volume* of 0.5 per cent

¹ If the solution is alkaline in reaction, while it is being concentrated, the amino acids will be broken down and ammonia will be liberated.

Na_2CO_3 .) In each of four tubes place 5 c.c. of *alkaline* pancreatic extract. Immerse one tube in cold water from the faucet, keep a second at room temperature and place a third on the water-bath at 40°C . Boil the contents of the fourth for a few moments, then cool and also keep it at 40°C . Into each tube introduce a small piece of fibrin and note the progress of digestion. In which tube does the most rapid digestion occur? What is the reason?

3. **Influence of Metallic Salts, etc.**—Prepare a series of tubes and into each tube place 6 volumes of water, 3 volumes of alkaline pancreatic extract and 1 volume of one of the chemicals listed in Experiment 18 under Salivary Digestion, page 40.

Introduce a small piece of fibrin into each of the tubes and keep them at 40°C . for one-half hour. Shake the tubes frequently. In which tubes do we get the least digestion?

4. **Influence of Bile.**—Prepare five tubes as follows:

- (a) Five c.c. of pancreatic extract + $\frac{1}{2}$ –1 c.c. of bile.
- (b) Five c.c. of pancreatic extract + 1–2 c.c. of bile.
- (c) Five c.c. of pancreatic extract + 2–3 c.c. of bile.
- (d) Five c.c. of pancreatic extract + 5 c.c. of bile.
- (e) Five c.c. of pancreatic extract.

Introduce into each tube a small piece of fibrin and keep them at 40°C . Shake the tubes frequently and note the progress of digestion. Does the presence of bile retard tryptic digestion? How do these results agree with those obtained under gastric digestion?

EXPERIMENTS ON AMYLOPSIN.

1. **The Most Favorable Reaction.**—Prepare seven tubes as follows:

- (a) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of water.
- (b) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 1 per cent Na_2CO_3 .
- (c) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 0.5 per cent Na_2CO_3 .

(d) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 0.2 per cent HCl.

(e) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 0.2 per cent *combined* HCl.

(f) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 0.4 per cent boric acid.

(g) One c.c. of *neutral* pancreatic extract + 1 c.c. of starch paste + 2 c.c. of 0.4 per cent acetic acid.

Shake each tube thoroughly and place them on the water-bath at 40° C. At the end of a half-hour divide the contents of each tube into two parts and test one part by the iodine test and the other part by Fehling's test. Where do you find the most satisfactory digestion? How do the results here compare with those obtained from the similar series under Trypsin, page 111.

2. **The Most Favorable Temperature.**—(For this and the following series of experiments upon amylopsin use the *neutral* extract plus an equal volume of 0.5 per cent Na_2CO_3 .) In each of four tubes place 2–3 c.c. of *alkaline* pancreatic extract. Immerse one tube in cold water from the faucet, keep a second at room temperature and place a third on the water-bath at 40° C. Boil the contents of the fourth for a few moments, then cool and also keep it at 40° C. Into each tube introduce 2–3 c.c. of starch paste and note the progress of digestion. At the end of one-half hour divide the contents of each tube into two parts and test one part by the iodine test and the other part by Fehling's test. In which tube do you find the most satisfactory digestion? How does this result compare with the result obtained in the similar series of experiments under Trypsin (see page 111)?

3. **Influence of Metallic Salts, etc.**—Prepare a series of tubes and into each tube place 3 volumes of water, 3 volumes of *alkaline* pancreatic extract, 1 volume of one of the chemicals listed in Experiment 18 under Salivary Digestion, page 40, and 3 volumes of starch paste. Be sure to introduce the *starch paste* into the tube *last*. Why? Shake the tubes well and

place them on the water-bath at 40° C. At the end of a half-hour divide the contents of each tube into two parts and test one part by the iodine test and the other part by Fehling's test. What are your conclusions?

4. **Influence of Bile.**—Prepare five tubes as follows:

(a) 2-3 c.c. of pancreatic extract + 2-3 c.c. of starch paste + $\frac{1}{2}$ -1 c.c. of bile.

(b) 2-3 c.c. of pancreatic extract + 2-3 c.c. of starch paste + 1-2 c.c. of bile.

(c) 2-3 c.c. of pancreatic extract + 2-3 c.c. of starch paste + 2-3 c.c. of bile.

(d) 2-3 c.c. of pancreatic extract + 2-3 c.c. of starch paste + 5 c.c. of bile.

(e) 2-3 c.c. of pancreatic extract + 2-3 c.c. of starch paste.

Shake the tubes thoroughly and place them on the water-bath at 40° C. Note the progress of digestion frequently and at the end of a half-hour divide the contents of each tube into two parts and test one part by the iodine test and the other part by Fehling's test. What are your conclusions regarding the influence of bile upon the action of amylopsin?

5. **Digestion of Dry Starch.**—To a little *dry* starch in a test-tube add about 5 c.c. of pancreatic extract and place the tube on the water-bath at 40° C. At the end of a half hour filter and test separate portions of the filtrate by the iodine and Fehling tests. What do you conclude regarding the action of amylopsin upon dry starch? Compare this result with that obtained in the similar experiment under Salivary Digestion (page 38).

6. **Digestion of Inulin.**—To 5 c.c. of inulin solution in a test-tube add 10 drops of pancreatic extract and place the tube on the water-bath at 40° C. After one-half hour test the solution by Fehling's test.¹ Is any reducing substance present? What do you conclude regarding the digestion of inulin by amylopsin?

¹ If the inulin solution gives a reduction before being acted upon by the pancreatic juice, it will be necessary to determine the extent of the original reduction by means of a "check" test (see page 26).

EXPERIMENTS ON STEAP SIN.

1. "**Litmus-Milk**" Test.—Into each of two test-tubes introduce 10 c.c. of milk and a small amount of litmus powder. To the contents of one tube add 3 c.c. of *neutral* pancreatic extract and to the contents of the other tube add 3 c.c. of water or of *boiled* neutral pancreatic extract. Keep the tubes at 40° C. and note any changes which may occur. What is the result and how do you explain it?

2. **Ethyl Butyrate Test**.—Into each of two test-tubes introduce 4 c.c. of water, 2 c.c. of ethyl butyrate, $C_3H_7COO \cdot C_2H_5$, and a small amount of litmus powder. To the contents of one tube add 4 c.c. of *neutral* pancreatic extract and to the contents of the other tube add 4 c.c. of water or *boiled* neutral pancreatic extract. Keep the tubes at 40° C. and observe any changes which may occur. What is the result and how do you explain it? Write the equation for the reaction which has taken place.

EXPERIMENTS ON PANCREATIC RENNIN.

Prepare two test-tubes as follows:

- (a) Five c.c. of milk + 10 drops of pancreatic extract.
- (b) Five c.c. of milk + 20 drops of pancreatic extract.

Place the tubes at 40°–45° C. for a half hour *without shaking*. Note the formation of a clot.¹ How does the action of pancreatic rennin compare with the action of the gastric rennin?

¹ This reaction will not always succeed, owing to conditions which are not well understood.

CHAPTER VIII.

BILE.

The bile is secreted continuously by the liver and passes into the intestine through the common bile duct which opens near the pylorus. Bile is *not* secreted continuously *into the intestine*. In a fasting animal no bile enters the intestine, but when food is taken the bile begins to flow; the length of time elapsing between the ingestion of the food and the secretion of the bile as well as the qualitative and quantitative characteristics of the secretion depending upon the nature of the food ingested. Fats, the extractives of meat and the end-products of gastric digestion (acid albuminate, proteoses and peptones) cause a copious secretion of bile, whereas such substances as water, acids and boiled starch paste fail to do so. In general a rich proteid diet is supposed to increase the amount of bile secreted, whereas a carbohydrate diet would rather tend to decrease the amount.

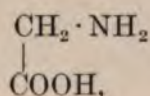
We may look upon the bile as an *excretion* as well as a *secretion*. In the fulfillment of its excretory function it passes such bodies as lecithin, metallic substances and cholesterin into the intestine and in this way aids in removing them from the organism. The bile assists materially in the absorption of fats from the intestine by its solvent action on the fatty acids formed by the action of the pancreatic juice.

The bile is a very thick, viscid substance which is alkaline in reaction to litmus and ordinarily possesses a decidedly bitter taste. It varies in color in the different animals, the principal variations being yellow, brown and green. Fresh human bile ordinarily has a green or golden-yellow color. Post-mortem bile is variable in color. It is very difficult to determine accurately the amount of normal bile secreted during any given period. For an adult man it has been variously

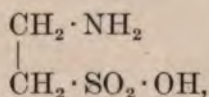
estimated at from 500 c.c. to 1100 c.c. for twenty-four hours. The specific gravity of the bile varies between 1.010 and 1.040, and the freezing-point is about -0.56°C . As secreted by the liver, the bile is a clear, limpid fluid which contains a relatively low content of solid matter. Such bile would have a specific gravity of approximately 1.010. After it reaches the gall-bladder, however, it becomes mixed with mucous material from the walls of the gall-bladder, and this process coupled with the continuous absorption of water from the bile has a tendency to concentrate the secretion. Therefore the bile, as we find it in the gall-bladder, ordinarily possesses a higher specific gravity than that of the freshly secreted fluid. The specific gravity under these conditions may run as high as 1.040.

The principal constituents of the bile are the *salts of the bile acids, bile pigments, neutral fats, lecithin and cholesterin*, besides the salts of *iron, copper, calcium and magnesium*. Zinc has also frequently been found in traces.

The bile acids, which are elaborated exclusively by the hepatic cells, may be divided into two groups, the *glycocholic acid* group and the *taurocholic acid* group. In human bile glycocholic acid predominates, while taurocholic acid is the more abundant in the bile of carnivora. The bile acids are conjugate *amino-acids*, the glycocholic acid yielding *glycocoll*,



and *cholic acid* upon decomposition, whereas taurocholic acid gives rise to *taurin*,



and *cholic acid* under like conditions. Glycocholic acid contains some nitrogen but no sulphur, whereas taurocholic acid contains both these elements. The sulphur of the taurocholic

acid is present in the taurin (amino-ethyl-sulphonic acid), of which it is a characteristic constituent. There are several varieties of cholic acid and therefore we have several forms of glycocholic and taurocholic acids, the variation in constitution depending upon the nature of the cholic acid which enters into the combination. The bile acids are present in the bile as salts of one of the alkalis, generally sodium. The sodium glycocholate and sodium taurocholate may be isolated in crystalline form, either as balls or rosettes of fine needles or in the form of prisms having ordinarily four or six sides (Fig. 40, below). The salts of the bile acids are dextro-rotatory. Among other properties these salts have the power of holding the cholesterin and lecithin of the bile in solution.

FIG. 40.



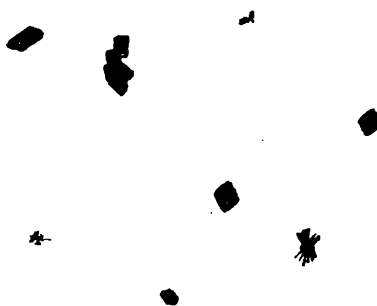
BILE SALTS.

The bile pigments are important and interesting biliary constituents. The following have been isolated: *bilirubin*, *biliverdin*, *bilifuscin*, *biliprasin*, *bilihumin*, *bilicyanin* and *choletelin*. Of these, bilirubin and biliverdin are the most important and predominate in normal bile. Bilirubin may be isolated as a reddish-yellow powder and biliverdin may be obtained in the form of a green powder. The colors possessed by the various

varieties of normal bile are due almost entirely to these two pigments, the biliverdin being the predominant pigment in greenish bile and the bilirubin being the principal pigment in lighter colored bile. The pigments, other than the two just mentioned, have been found almost exclusively in biliary calculi or in altered bile obtained at post-mortem examinations.

Bilirubin, which is perhaps the most important of the bile pigments, is apparently derived from the blood pigment, the iron freed in the process being held in the liver. Bilirubin has the same percentage composition as hæmatoporphyrin, which may be produced from hæmatin. It is a specific product of the liver cells but may also be formed in other parts of the body. The pigment may be obtained, in part, in the form of reddish-yellow rhombic plates (Fig. 41, below) upon the

FIG. 41.

BILIRUBIN (HÆMATOIDIN). (*Ogden.*)

spontaneous evaporation of its chloroform solution. The crystalline form of bilirubin is practically the same as that of hæmatoidin. It is easily soluble in chloroform, somewhat less soluble in alcohol and only slightly soluble in ether and benzene. Bilirubin has the power of combining with certain metals, particularly calcium, to form combinations which are no longer soluble in the solvents of the unaltered pigment. Upon long standing in contact with the air, the reddish-yellow bilirubin is oxidized with the formation of the green biliverdin. Bilirubin occurs in animal fluids as soluble bilirubin-alkali.

Solutions of bilirubin exhibit no absorption-bands. If an ammoniacal solution of bilirubin-alkali in water is treated with a solution of zinc chloride, however, it shows bands similar to those of bilicyanin (Absorption Spectra, Plate II), the two bands between C and D being rather well defined.

Biliverdin is particularly abundant in the bile of herbivora. It is soluble in alcohol and glacial acetic acid and insoluble in water, chloroform and ether. Biliverdin is formed from bilirubin upon oxidation. It is an amorphous substance, and in this differs from bilirubin which may be at least partly crystallized under proper conditions. In common with bilirubin, it may be converted into hydrobilirubin by nascent hydrogen.

The neutral solution of bilicyanin or cholecyanin is bluish-green or steel-blue and possesses a blue fluorescence, the alkaline solution is green with no appreciable fluorescence and the strongly acid solution is violet-blue. The alkaline solution exhibits three absorption-bands, the first a dark, well-defined band between C and D somewhat nearer C; the second a less sharply-defined band extending across D and the third a rather faint band between E and F, near E (Absorption Spectra, Plate II). The strongly acid solution exhibits two absorption bands, both lying between C and E and separated by a narrow space near D. A third band, exceedingly faint, may ordinarily be seen between b and F.

Biliary calculi, otherwise designated as *biliary concretions* or *gall stones*, are frequently formed in the gall-bladder. These deposits may be divided into three classes, *cholesterin calculi*, *pigment calculi* and calculi made up almost entirely of *inorganic material*. This last class of calculus is formed principally of the carbonate and phosphate of calcium and is rarely found in man although quite common to cattle. The pigment calculus is also found in cattle, but is more common to man than the inorganic calculus. This pigment calculus ordinarily consists principally of bilirubin in combination with calcium; biliverdin is sometimes found in small amount. The

cholesterin calculus is the one found most frequently in man. These may be formed almost entirely of cholesterin, in which event the color of the calculus is very light, or they may contain more or less pigment and inorganic matter mixed with the cholesterin, which tends to give us calculi of various colors.

For discussion of cholesterin see page 222.

EXPERIMENTS ON BILE.

1. **Reaction.**—Test the reaction of fresh ox bile to litmus.
2. **Nucleo-proteid.**—Acidify a small amount of bile with dilute acetic acid. A precipitate of nucleo-proteid forms.

3. **Inorganic Constituents.**—Test for chlorides, sulphates and phosphates (see page 37).

4. **Tests for Bile Pigments.** (a) *Gmelin's Test.*—To about 5 c.c. of *concentrated* nitric acid in a test-tube add 2–3 c.c. of diluted bile *carefully* so that the two fluids do not mix. At the point of contact note the various colored rings, *green, blue, violet, red* and *reddish-yellow*. Repeat this test with different dilutions of bile and observe its delicacy.

(b) *Rosenbach's Modification of Gmelin's Test.*—Filter 5 c.c. of diluted bile through a small filter paper. Introduce a drop of *concentrated* nitric acid into the cone of the paper and note the succession of colors as given in Gmelin's test.

(c) *Huppert's Reaction.*—Thoroughly shake equal volumes of undiluted bile and milk of lime in a test-tube. The pigments unite with the calcium and are precipitated. Filter off the precipitate, wash it with water and transfer to a small beaker. Add alcohol acidified slightly with hydrochloric acid and warm upon a water-bath until the solution becomes colored an emerald green.

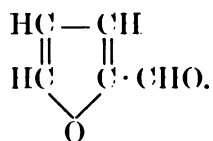
(d) *Hammarsten's Reaction.*—To about 5 c.c. of Hammarsten's reagent¹ in a small evaporating dish add a few drops of diluted bile. A green color is produced. If more of the reagent is now added the play of colors as observed in Gmelin's test may be obtained.

¹ Hammarsten's reagent is made by mixing 1 volume of 25 per cent nitric acid and 19 volumes of 25 per cent hydrochloric acid and then adding 1 volume of this acid mixture to 4 volumes of 95 per cent alcohol.

(e) *Smith's Test*.—To 2–3 c.c. of diluted bile in a test-tube add *carefully* about 5 c.c. of dilute tincture of iodine (1 : 10) so that the fluids do not mix. A play of colors, *green*, *blue* and *violet*, is observed. In making this test upon the urine ordinarily only the *green* color is observed. Try the test upon various dilutions of bile and note its delicacy as compared with that of Gmelin's test. Which test do you consider the more delicate?

5. **Tests for Bile Acids.** (a) *Pettenkofer's Test*.—To 5 c.c. of diluted bile in a test-tube add 5 drops of a 5 per cent solution of saccharose. Now run about 2–3 c.c. of concentrated sulphuric acid *carefully* down the side of the tube and note the *red* ring at the point of contact. Upon slightly agitating the contents of the tube the whole solution gradually assumes a *reddish* color. As the tube becomes warm, it should be cooled in running water in order that the temperature of the solution may not rise above 70° C.

(b) *Mylius's Modification of Pettenkofer's Test*. To approximately 5 c.c. of diluted bile in a test-tube add 3 drops of a very dilute (1 : 1,000) aqueous solution of furfural,



Now run about 2–3 c.c. of concentrated sulphuric acid *carefully* down the side of the tube and note the *red* ring as above. In this case also, upon shaking the tube, the whole solution is colored red. Keep the temperature of the solution below 70° C. as before.

(c) *Neukomm's Modification of Pettenkofer's Test*.—To a few drops of diluted bile in an evaporating dish add a trace of a dilute saccharose solution and one or more drops of dilute sulphuric acid. Evaporate on a water-bath and note the development of a *violet* color at the edge of the evaporating

mixture. Discontinue the evaporation as soon as the color is observed.

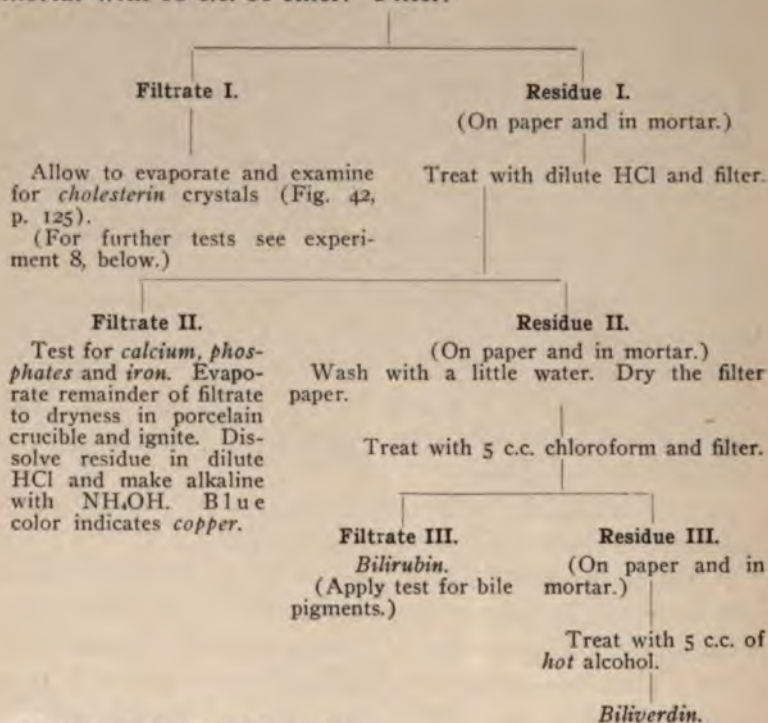
(d) *v. Udránsky's Test*.—To 5 c.c. of diluted bile in a test-tube add 3–4 drops of a very dilute (1:1,000) aqueous solution of furfurol. Place the thumb over the top of the tube and shake the tube until a thick foam is formed. By means of a small pipette add 2–3 drops of concentrated sulphuric acid to the foam and note the *dark pink* coloration produced.

(e) *Hay's Test*.—Cool about 10 c.c. of diluted bile in a test-tube to 17° C. or lower and sprinkle a little finely pulverized sulphur upon the surface of the fluid. The presence of bile acids is indicated if the sulphur sinks to the bottom of the liquid, the rapidity with which the sulphur sinks depending upon the quantity of bile acids present in the mixture. The test is said to react with bile acids when they are present in the proportion 1:120,000.

Some investigators claim that it is impossible to differentiate between bile acids and bile pigments by this test.

6. **Crystallization of Bile Salts**.—To 25 c.c. of *undiluted* bile in an evaporating dish add enough animal charcoal to form a paste and evaporate to dryness on a water-bath. Remove the residue, grind it in a mortar and transfer it to a small flask. Add about 50 c.c. of 95 per cent alcohol and boil on a water-bath for 20 minutes. Filter, and add ether to the filtrate until there is a slight *permanent* cloudiness. Cover the vessel and stand it away until crystallization is complete. Examine the crystals under the microscope and compare them with those shown in Fig. 40, page 118. Try one of the tests for bile acids upon some of the crystals.

7. **Analysis of Biliary Calculi.**—Grind the calculus in a mortar with 10 c.c. of ether. Filter.



8. Tests for Cholesterin.

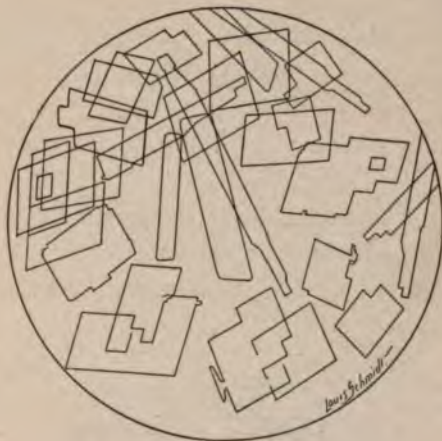
(a) *Microscopical Examination.*—Examine the crystals under the microscope and compare them with those shown in Fig. 42, page 125.

(b) *Iodine-Sulphuric Acid Test.*—Place a few crystals of cholesterin in one of the depressions of a test-tablet and treat with a drop of concentrated sulphuric acid and a drop of a very dilute solution of iodine. A play of colors consisting of *violet*, *blue*, *green* and *red* results.

(c) *The Liebermann-Burchard Test.*—Dissolve a few crystals of cholesterin in 2 c.c. of chloroform in a dry test-tube. Now add 10 drops of acetic anhydride and 1–3 drops of concentrated sulphuric acid. The solution becomes *red*, then *blue*, and finally *bluish-green* in color.

(d) *Salkowski's Test*.—Dissolve a few crystals of cholesterol in a little chloroform and add an equal volume of concentrated sulphuric acid. A play of colors from *bluish-red* to *cherry-red* and *purple* is noted in the chloroform while the acid assumes a marked green fluorescence.

FIG. 42.



CHOLESTERIN.

(e) *Schiff's Reaction*.—To a little cholesterol in an evaporating dish add a few drops of a mixture of 3 volumes of concentrated sulphuric acid and 1 volume of 10 per cent ferric chloride. Evaporate to dryness over a low flame and observe the *reddish-violet* residue which changes to a *bluish-violet*.

9. **Preparation of Taurin**.—To 300 c.c. of bile in a casserole add 100 c.c. of hydrochloric acid and heat until a sticky mass (dyslysin) is formed. This point may be determined by drawing out a thread-like portion of the mass by means of a glass rod, and if it solidifies immediately and assumes a brittle character we may conclude that all the taurocholic and glycocholic acid has been decomposed. Decant the solution and concentrate it to a small volume on the water-bath. Filter the hot solution to remove sodium chloride and other substances which may have separated, and evaporate the filtrate to dry-

ness. Dissolve the residue in 5 per cent hydrochloric acid and precipitate with ten volumes of 95 per cent alcohol. Filter off the taurin and recrystallize it from hot water. (Save the alcoholic filtrate for the preparation of glycocoll, page 127.) Make the following tests upon the taurin crystals:

(a) Examine them under the microscope and compare with Fig. 43, below.

(b) Heat a crystal upon platinum foil. The taurin at first melts, then turns brown and finally carbonizes as the temperature is raised. Note the suffocating odor. What is it?

(c) Test the solubility of the crystals in water and in alcohol.

(d) Grind up a crystal with four times its volume of dry sodium carbonate and fuse on platinum foil. Cool the residue, transfer it to a test-tube and dissolve it in water. Add a little

FIG. 43.



TAURIN.

dilute sulphuric acid and note the odor of hydrogen sulphide. Hold a piece of filter paper, moistened with a *small* amount of lead acetate, over the opening of the test-tube and observe the formation of lead sulphide.

10. **Preparation of Glycocoll.**—Concentrate the alcoholic filtrate from the last experiment (9) until no more alcohol remains. The glycocoll is present here in the form of an hydrochloride and may be liberated from this combination by the addition of freshly precipitated lead hydroxide or by lead hydroxide solution. Remove the lead by H_2S . Filter and decolorize the filtrate by animal charcoal. Filter again, concentrate the filtrate and set it aside for crystallization. Glycocoll separates as colorless crystals (Fig. 44, below).

FIG. 44.

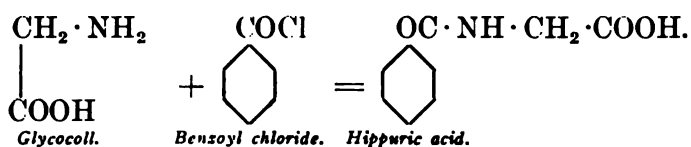


GLYCOCOLL.

11. **Synthesis of Hippuric Acid.**—To some of the glycocoll prepared in the last experiment or furnished by the instructor, add a little water, about 1 c.c. of benzoyl chloride and render alkaline with potassium hydroxide solution. Stopper the tube and shake it until no more heat is evolved. Now render strongly alkaline with potassium hydroxide and shake the mixture until no odor of benzoyl chloride can be detected. Cool, acidify with HCl , add an equal volume of petroleum ether (ethyl ether may be substituted) and shake thoroughly to remove the benzoic acid. (Evaporate this solution and note the crystals of benzoic acid. Compare them with those shown

in Fig. 94, page 264.) Decant the ethereal solution into a porcelain dish and extract again with ether. The hippuric acid remains in the aqueous solution. Filter it off and wash it with a small amount of cold water while still on the filter. Remove it to a small shallow vessel, dissolve it in a small amount of hot water and set it aside for crystallization. Examine the crystals microscopically and compare them with those in Fig. 92, page 256.

The chemistry of the synthesis is represented thus :

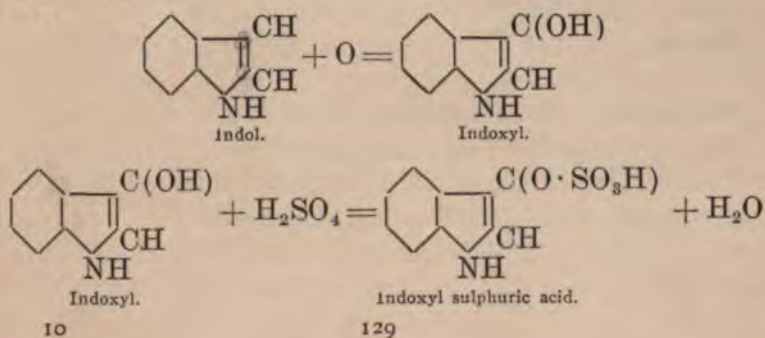


CHAPTER IX.

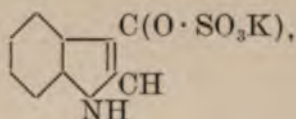
PUTREFACTION PRODUCTS.

The putrefactive processes in the intestine are the result of the action of bacteria upon the proteid material present. This bacterial action which is the combined effort of many forms of micro-organisms is confined almost exclusively to the large intestine. Some of the products of the putrefaction of proteids are identical with those formed in tryptic digestion although the decomposition of the proteid material is much more extensive when subjected to putrefaction. Some of the more important of the putrefaction products are the following: *Indol, skatol, paracresol, phenol, para-oxyphenylpropionic acid, para-oxyphenylacetic acid, volatile fatty acids, hydrogen sulphide, methane, methyl mercaptan, hydrogen, and carbon dioxide*, beside *proteoses, peptones, ammonia and amino acids*. Of these the indol, skatol and phenol appear in part in the urine as ethereal sulphuric acids, whereas the oxyacids mentioned pass unchanged into the urine. The potassium indoxyl sulphate (page 130) content of the urine is a rough indicator of the extent of the putrefaction within the intestine.

The portion of the indol which is excreted in the urine is first subjected to a series of changes within the organism and is subsequently eliminated as *indican*. These changes may be represented thus:

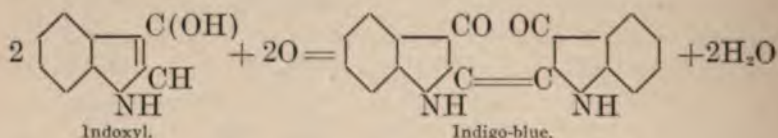


In the presence of potassium salts the indoxyl sulphuric acid is then transformed into potassium indoxyl sulphate (or indican),



and eliminated as such in the urine.

Indican may be decomposed by treatment with concentrated hydrochloric acid (see tests on page 255) into sulphuric acid and indoxyl. The latter body may then be oxidized to form indigo-blue thus:



Skatol is likewise changed within the organism and eliminated in the form of a chromogenic substance.

EXPERIMENTS ON PUTREFACTION PRODUCTS.

In many courses in physiological chemistry the instructors are so limited for time that no extended study of the products of putrefaction can very well be attempted. Under such conditions the scheme here submitted may be used profitably in the way of a demonstration. Where the number of students is not too great, a single large putrefaction may be started, and, after the initial distillation, both the resulting distillate and residue may be distributed to the members of the class for individual manipulation.

Preparation of Putrefaction Mixture.—Place a weighed mixture of coagulated egg albumin and ground lean meat in a flask or bottle and add approximately 2 liters of water for every kilogram of proteid used. Sterilize the vessel and contents, inoculate with the *colon bacillus* and keep at 40° C. for two or three weeks. If cultures of the *colon bacillus* are not

available, add 60 c.c. of a cold saturated solution of sodium carbonate for every liter of water previously added and inoculate with some putrescent material (pancreas or feces).¹ Mix the putrefaction mixture very thoroughly by shaking and insert a cork furnished with a glass tube to which is attached a wash bottle containing a 3 per cent solution of mercuric cyanide.² This device is for the purpose of collecting the methyl mecaptan, a gas formed during the process of putrefaction. It also serves to diminish the odor arising from the putrefying material. Place the putrefaction mixture at 40° C. for two or three weeks and at the end of that time make a separation of the products of putrefaction according to the following directions:

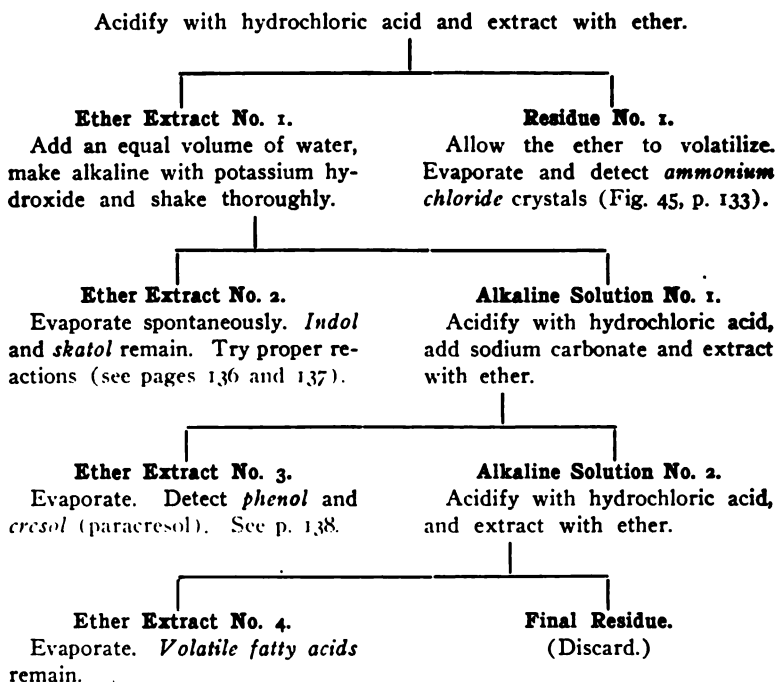
Subject the mixture to distillation until the distillate and residue are approximately equal in volume.

¹ Putrefying proteid may be prepared by treating 10 grams of finely ground lean meat with 100 c.c. of water and 2 c.c. of a saturated solution of sodium carbonate and keeping the mixture at 40° C. for twenty-four hours.

² Concentrated sulphuric acid containing a small amount of *isatin* may be used as a substitute for mercuric cyanide. When this modification is employed it is necessary to use calcium chloride tubes to exclude moisture from the *isatin* solution.

PART I.

MANIPULATION OF THE DISTILLATE.



DETAILED DIRECTIONS FOR MAKING THE
SEPARATIONS INDICATED IN
THE SCHEME.

Preliminary Ether Extraction.—This extraction may be conveniently conducted in a separatory funnel. Mix the fluids for extraction in the ratio of *two* volumes of ether to *three* volumes of the distillate. Shake very thoroughly for a few moments, then draw off the extracted fluid and add a new portion of the distillate. Repeat the process until the entire distillate has been extracted. Add a small amount of fresh

ether at each extraction to replace that dissolved by the water in the preceding extraction.

Residue No. 1.—Unite the portions of the distillate extracted as above and allow the ether to volatilize spontaneously.

FIG. 45.



AMMONIUM CHLORIDE.

Evaporate until crystallization begins. Examine the crystals under the microscope. Ammonium chloride predominates. Explain its presence.

Ether Extract No. 1.—Add an equal volume of water, render the mixture alkaline with potassium hydroxide and shake thoroughly by means of a separatory funnel as before. The *volatile fatty acids*, contained among the putrefaction products, would be dissolved by the alkaline solution (No. 1) whereas any *indol* or *skatol* would remain in the ethereal solution (No. 2).

Alkaline Solution No. 1.—Acidify with hydrochloric acid and add sodium carbonate solution until the fluid is neutral or slightly acid from the presence of carbonic acid. At this point a portion of the solution, after being heated for a few moments, should possess an alkaline reaction on cooling. Extract the whole mixture with ether in the usual way, using care in the

manipulation of the stop cock to relieve the pressure due to the evolution of carbon dioxide. The ether (Ether Extract No. 3) removes any *phenol* or *cresol* which may be present while the volatile fatty acids will remain in the alkaline solution (No. 2) as alkali salts.

Ether Extract No. 2.—Drive off the major portion of the ether at a low temperature on a water-bath and allow the residue to evaporate spontaneously. Indol and skatol should be present here. Prove the presence of these bodies. For tests for indol and skatol see p. —.

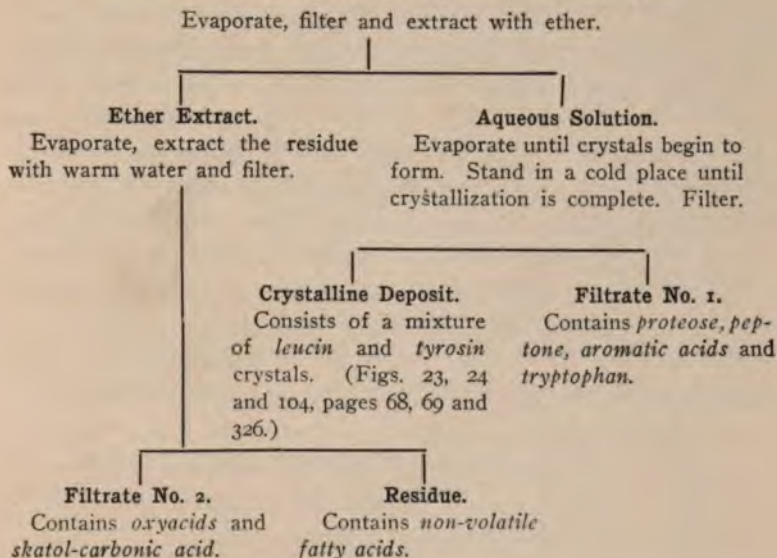
Alkaline Solution No. 2.—Make strongly acid with hydrochloric acid and extract with a small amount of ether, using a separatory funnel. As carbon dioxide is liberated here, care must be used in the manipulation of the stop cock of the funnel in relieving the pressure within the vessel. The volatile fatty acids are dissolved by the ether (Ether Extract No. 4).

Ether Extract No. 3.—Evaporate this ethereal solution on a water-bath. The oily residue contains phenol and cresol. The cresol is present for the most part as paracresol. Add some water to the oily residue and heat it in a flask. Cool and prove the presence of phenol and cresol. For tests for these bodies see page 138.

Ether Extract No. 4.—Evaporate on a water-bath. The volatile fatty acids remain in the residue.

PART II.

MANIPULATION OF THE RESIDUE.



DETAILED DIRECTIONS FOR MAKING THE SEPARATIONS INDICATED IN THE SCHEME.

Preliminary Ether Extraction.—This extraction may be conducted in a separatory funnel. In order to make a satisfactory extraction the mixture should be shaken very thoroughly. Separate the ethereal solution from the aqueous portion and treat them according to the directions given below.

Ether Extract.—Evaporate this solution on a *safety* water-bath until the ether has been entirely removed. Extract the residue with warm water and filter.

Aqueous Solution.—Evaporate this solution until crystallization begins. Stand the solution in a cold place until no more crystals form. This crystalline mass consists of impure *leucin* and *tyrosin*. Filter off the crystals.

Crystalline Deposit.—Examine the crystals under the microscope and compare them with those reproduced in Figs. 23, 24 and 104, pages 68, 69 and 326. Do the forms of the crystals of leucin and tyrosin resemble those previously examined? Make a separation of the leucin and tyrosin and apply typical tests according to directions given on pages 81 and 82.

Filtrate No. 1.—Make a test for tryptophan with bromine water (see page 110), and, also with the Hopkins-Cole reagent (see page 45). Use the remainder of the filtrate for the separation of proteoses and peptones. Make the separation according to the directions given on page 59.

Filtrate No. 2.—This solution contains para-oxyphenylacetic acid, para-oxyphenylpropionic acid and skatol-carbonic acid. Prove the presence of these bodies by appropriate tests. Tests for oxyacids and skatol-carbonic acid are given on page 138.

TESTS FOR VARIOUS PUTREFACTION PRODUCTS.

Tests for Indol.

1. **Herter's Naphthaquinone Reaction.**—(a) To a dilute aqueous solution of indol (1:50,000) add one drop of a 2 per cent solution of naphthaquinone sodium-monosulphonate. No reaction occurs. Add a drop of a 10 per cent solution of potassium hydroxide and note the gradual development of a blue or blue-green color which fades to green if an excess of the alkali is added. Render the green or blue-green solution acid and note the appearance of a pink color. Heat facilitates the development of the color reaction.

One part of indol in *one million parts* of water may be detected by means of this test if carefully performed.

(b) If the alkali be added to the indol solution *before* the introduction of the naphthaquinone the course of the reaction is different, particularly if the indol solution is somewhat more concentrated than that mentioned above and if heat is used. Under these conditions the blue indol compound ultimately forms as fine acicular crystals which rise to the surface.

If we do not wait for the production of the crystalline body but as soon as the blue color forms, shake the aqueous solution with chloroform, the blue color disappears from the solution and the chloroform assumes a *pinkish-red hue*. This is a distinguishing feature of the indol reaction and facilitates the differentiation of indol from other bodies which yield a similar blue color.

2. **Cholera-red Reaction.**—To a little of the residue in a test-tube add one-tenth its volume of a 0.02 per cent solution of potassium nitrite and mix thoroughly. Carefully run concentrated sulphuric acid down the side of the tube so that it forms a layer at the bottom. Note the purple color. Neutralize with potassium hydroxide and observe the production of a bluish-green color.

3. **Legal's Reaction.**—To a small amount of the residue in a test-tube add a few drops of a freshly prepared solution of sodium nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} + 2\text{H}_2\text{O}$. Render alkaline with potassium hydroxide and note the production of a violet color. If the solution is now acidified with glacial acetic acid the violet is transformed into a blue.

4. **Pine Wood Test.**—Moisten a pine splinter with concentrated hydrochloric acid and insert it into the residue. The wood assumes a cherry-red color.

5. **Nitroso-indol Nitrate Test.**—Acidify some of the residue with nitric acid, add a few drops of a potassium nitrite solution and note the production of a red precipitate of nitroso-indol nitrate. If the residue contains but little indol simply a red coloration will result. Compare this result with the result of the similar test on skatol.

Tests for Skatol.

1. **Herter's Naphthaquinone Reaction.**—The same procedure may be used here as in the similar test under indol, page 136. The distinctive feature of dilute solutions of skatol when treated with the naphthaquinone compound is that they yield a *violet or purple instead of a blue*. Concentrated solu-

tions of skatol yield the blue color as noted with indol. This reaction possesses relatively the same delicacy as the indol reaction.

2. **Color Reaction with HCl.**—Acidify some of the residue with concentrated hydrochloric acid. Note the production of a violet color.

3. Acidify some of the residue with nitric acid and add a few drops of a potassium nitrite solution. Note the white turbidity. Compare this result with the result of the similar test on indol.

Tests for Phenol and Cresol.

1. **Color Test.**—Test a little of the solution with Millon's reagent. A red color results. Compare this test with the similar one under Tyrosin (see page 82).

2. **Ferric Chloride Test.**—Add a few drops of *neutral* ferric chloride solution to a little of the residual fluid. A dirty bluish-gray color is formed.

3. **Formation of Bromine Compounds.**—Add some bromine water to a little of the fluid under examination. Note the crystalline precipitate of tribromphenol and tribromcresol.

Tests for Oxyacids.

1. **Color Test.**—Test a little of the solution with Millon's reagent. A red color results.

2. **Bromine Water Test.**—Add a few drops of bromine water to some of the filtrate. A turbidity or precipitate is observed.

Test for Skatol-carbonic Acid.

Ferric Chloride Test.—Acidify some of the filtrate with hydrochloric acid, add a few drops of ferric chloride solution and heat. Compare the end-reaction with that given by phenol.

CHAPTER X.

FECES.

The feces is the residual mass of material remaining in the intestine after the full and complete exercise of the digestive and absorptive functions and is ultimately expelled from the body through the rectum. The amount of this fecal discharge varies with the individual and the diet. Upon an ordinary mixed diet the daily excretion by an adult male will aggregate 110-170 grams with a solid content ranging between 25 and 45 grams; the fecal discharge of such an individual upon a

FIG. 46.



MICROSCOPICAL CONSTITUENTS OF FECES. (*v. Jaksch.*)

a, Muscle fibers; *b*, connective tissue; *c*, epithelium; *d*, leucocytes; *e*, spiral cells; *f*, *g*, *h*, *i*, various vegetable cells; *k*, "triple phosphate" crystals; *l*, woody vegetable cells; the whole interspersed with innumerable microorganisms of various kinds.

vegetable diet will be much greater and may even be as great as 350 grams and possess a solid content of 75 grams. The variation in the normal daily output being so great renders this factor of very little value for diagnostic purposes, except where the composition of the diet is accurately known.

Lesions of the digestive tract, a defective absorptive function or increased peristalsis as well as an admixture of mucus, pus, blood and pathological products of the intestinal wall may cause the total amount of excrement to be markedly increased.

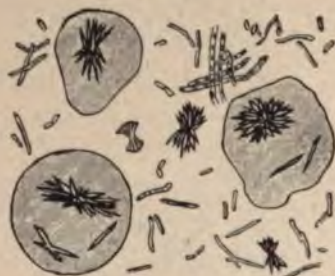
The fecal pigment of the normal adult is hydrobilirubin (urobilin or stercobilin). Neither bilirubin nor biliverdin occurs normally in the fecal discharge of adults, although the former may be detected in the excrement of nursing infants. The most important factor, however, in determining the color of the fecal discharge is the diet. A mixed diet for instance

produces stools which vary in color from light to dark brown, an exclusive meat diet gives rise to a brownish-black stool, whereas the stool resulting from a milk diet is invariably light colored. Certain pigmented foods such as the chlorophyllic vegetables, and various varieties of berries, each afford stools having a characteristic color. Certain drugs act in a similar way

to color the fecal discharge. This is well illustrated by the occurrence of green stools following the use of calomel and of black stools after bismuth ingestion. The green color of the calomel stool is generally believed to be due to biliverdin. v. Jaksch however claims to have proven this view to be incorrect since he was able to detect hydrobilirubin (or urobilin) but *no biliverdin* in stools after the administration of calomel. The bismuth stool derives its color from the black sulphide which is formed from the subnitrate of bismuth. In cases of biliary obstruction the grayish-white *acholic* stool is formed.

Under normal conditions the odor of feces is due to *skatol* and *indol*, two bodies formed in the course of putrefac-

FIG. 47.



HÆMATOIDIN CRYSTALS FROM ACHOLIC STOOLS. (v. Jaksch.)

Color of crystals same as the color of those in Fig. 41, p. 119.

tive processes occurring within the intestine (see page 129). Such bodies as methane, methyl mercaptan and hydrogen sulphide may also add to the disagreeable character of the odor. The intensity of the odor depends to a large degree upon the character of the diet, being very marked in stools from a meat diet, much less marked in stools from a vegetable diet and frequently hardly detectable in stools from a milk diet. Thus the stool of the infant is ordinarily nearly odorless and any decided odor may generally be readily traced to some pathological source.

A neutral reaction ordinarily predominates in normal stools although slightly alkaline or even acid stools are met with. The acid reaction is encountered much less frequently than the alkaline and then commonly only following a vegetable diet.

The form and consistency of the stool is dependent, in large measure, upon the nature of the diet and particularly upon the quantity of water ingested. Under normal conditions the consistency may vary from a thin, pasty discharge to a firmly formed stool. Stools which are exceedingly thin and watery ordinarily have a pathological significance. In general the feces of the carnivorous animals is of a firmer consistency than that of the herbivora.

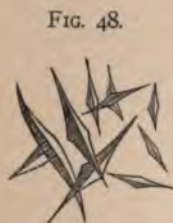


FIG. 48.
CHARCOT-LEYDEN
CRYSTALS.

Among the macroscopical constituents of the feces may be mentioned the following: Intestinal parasites, undigested food particles, gall stones, pathological products of the intestinal wall, enteroliths, intestinal sand and objects which have been accidentally swallowed.

The fecal constituents which at various times and under different conditions may be detected by the use of the microscope are as follows: Constituents derived from the food, such as *muscle fibers*, *connective tissue shreds*, *starch granules* and *fat*; formed elements derived from the intestinal tract, such as *epithelium*, *erythrocytes* and *leucocytes*; *mucus*; *pus corpuscles*; *parasites* and *bacteria*. In addition to the consti-

tients named, the following *crystalline deposits* may be detected: *Cholesterin, fatty acid, fat, bismuth sulphide, hæmatoidin, "triple phosphate," Charcot-Leyden crystals* and the *oxalate, carbonate, phosphate, sulphate* and *lactate* of calcium.

The detection of minute quantities of blood in the feces ("occult blood") has recently become a recognized aid to a correct diagnosis of certain disorders. In these instances the hemorrhage is ordinarily so slight that the identification by means of macroscopical characteristics as well as the microscopical identification through the detection of erythrocytes are both unsatisfactory in their results. Of the tests given for the detection of "occult blood" the *aloin-turpentine test* (page 144) is probably the most satisfactory. Since "occult blood" occurs with considerable regularity and frequency in gastrointestinal cancer and in gastric and duodenal ulcer, its detection in the feces is of especial value as an aid to a correct diagnosis of these disorders.

For diagnostic purposes the macroscopical and microscopical examinations of the feces ordinarily yield much more satisfactory data than are secured from its chemical examination.

EXPERIMENTS ON FECES.

1. **Macroscopical Examination.**—If the stool is watery pour it into a shallow dish and examine directly. If it is firm or pasty it should be treated with water and carefully stirred before the examination for macroscopical constituents is attempted.

The macroscopical constituents may be collected very satisfactorily by means of a Boas sieve (Fig. 49, page 143). This sieve is constructed of two easily detachable hemispheres which are held together by means of a bayonet catch. In using the apparatus the feces is spread out upon a very fine sieve contained in the lower hemisphere and a stream of water is allowed to play upon it through the medium of an opening in the upper hemisphere. The apparatus is provided with an orifice in the upper hemisphere through which the feces may be

stirred by means of a glass rod during the washing process. After 15–30 minutes washing nothing but the coarse fecal constituents remain upon the sieve.

2. **Microscopical Examination.**—Watery stools should be placed in a shallow dish, thoroughly mixed and a small amount

removed to a slide for examination. Stools of a firm or pasty consistency should be rubbed up in a mortar with water and a small portion of the resulting mixture transferred to a slide for examination. In normal feces look for *food particles*, *bacteria* and *crystalline bodies*. In pathological stools, in addition to these substances, look for *animal parasites* and *pathological products* of the intestinal wall.

3. **Reaction.**—Thoroughly mix the feces and apply moist red and blue litmus papers to the surface. If the stool is hard it should be mixed with water before the reaction is taken. Examine the stool as soon after defecation as is convenient, since the *reaction*

may change very rapidly. The *reaction* of the normal stools of adult man is ordinarily neutral or faintly alkaline to litmus, but seldom acid. Infants' stools are generally acid in reaction.

4. **Starch.**—If any imperfectly cooked starch-containing food has been ingested it will be possible to detect starch granules by a microscopical examination of the feces. If the granules are not detected by a microscopical examination, the feces should be placed in an evaporating dish or casserole and boiled with water for a few minutes. Filter and test the filtrate by the iodine test in the usual way (see page 24).

5. **Cholesterin and Fat.**—Extract the *dry* feces with ether in a Soxhlet apparatus (see Chapter XXII). If this apparatus is not available transfer the dry feces to a flask, add ether and shake frequently for a few hours. Filter and remove the

FIG. 49.



BOAS' SIEVE.

ether by evaporation. The residue contains cholesterin and the mixed fats of the feces. For every gram of fat add about $1\frac{1}{2}$ grams of solid potassium hydroxide and 25 c.c. of 95 per cent alcohol and boil in a flask on a water-bath for one-half hour maintaining the volume of alcohol constant. This alcoholic-potash has saponified the mixed fats and we now have a mixture of soaps and cholesterin. Add sodium chloride, in substance, to the mixture and extract with ether to dissolve out the cholesterin. Remove the ether by evaporation and examine the residue microscopically for cholesterin crystals. Try any of the other tests for cholesterin as given on page 124.

6. **Blood.**—Undecomposed blood may be detected macroscopically. If uncertain, look for erythrocytes under the microscope, and spectroscopically for the spectrum of oxyhæmoglobin (see Absorption Spectra, Plate I).

In case the blood has been altered or is present in minute amount ("occult blood"), and cannot be detected by the means mentioned above, the following tests may be tried:

(a) *Aloin-Turpentine Test.*—Mix the stool very thoroughly and take about 5 grams of the mixture for the test. Reduce this sample to a semi-fluid mass by means of distilled water and extract very thoroughly with an equal volume of ether to remove any fat which may be present. Now treat the extracted feces with one-third its volume of glacial acetic acid and 10 c.c. of ether and extract very thoroughly as before. The acid-ether extract will rise to the top and may be removed.

Introduce 2-3 c.c. of this acid-ether solution into a test-tube, add an equal volume of a dilute solution of aloin in 70 per cent alcohol and 2-3 c.c. of ozonized turpentine and shake the tube gently. If blood is present the entire volume of fluid ordinarily becomes pink and finally cherry red. In some instances the color will be limited to the aloin solution which sinks to the bottom. This color reaction should occur within fifteen minutes in order to indicate a positive test for blood, since the aloin will turn red of itself if allowed to stand for a longer period. The color is ordinarily light yellow in a nega-

tive test. Hydrogen peroxide is not a satisfactory substitute for turpentine in this test.

(b) *Weber's Guaiac Test*.—Mix a little feces with 30 per cent acetic acid to form a fluid mass. Transfer to a test-tube and extract with ether. If blood is present the ether will assume a brownish-red color. Filter off the ether extract and, to a portion of the filtrate, add an alcoholic solution of guaiac (strength about 1:60),¹ drop by drop, until the fluid becomes turbid. Now add hydrogen peroxide or old turpentine. In the presence of blood a blue color is produced (see page 158).

(c) *Acid-Hæmatin*.—Examine some of the ethereal extract from the last experiment (b) spectroscopically. Note the typical spectrum of acid-hæmatin (see Absorption Spectra, Plate II).

7. *Hydrobilirubin*.—Rub up a small amount of feces in a mortar with a concentrated aqueous solution of mercuric chloride. Transfer to a shallow flat-bottomed dish and allow to stand several hours. The presence of hydrobilirubin will be indicated by a deep red color imparted to the feces. This red color is due to the formation of hydrobilirubin-mercury. If unaltered bilirubin is present the feces will be green in color.

Another method for the detection of hydrobilirubin is the following: Treat the dry feces with absolute alcohol acidified with sulphuric acid and shake thoroughly. The acidified alcohol extracts the pigment and assumes a reddish color. Examine a little of this fluid spectroscopically and note the typical spectrum of hydrobilirubin (Absorption Spectra Plate II).

8. *Bilirubin*. (a) *Gmelin's Test*.—Place a few drops of concentrated nitric acid in an evaporating dish or on a porcelain test-tablet and allow a few drops of feces and water to mix with it. The usual play of colors of Gmelin's test is produced, *i. e.*, green, blue, violet, red and yellow. If so desired, this test may be executed on a slide and observed under the microscope.

¹ Buckmaster advises the use of an alcoholic solution of guaiaconic or of an alcoholic solution of guaiac resin.

(b) *Huppert's Test*.—Treat the feces with water to form a semi-fluid mass, add an equal amount of milk of lime, shake thoroughly and filter. Wash the precipitate with water, then transfer both the paper and the precipitate to a small beaker or flask, add a small amount of 95 per cent alcohol acidified slightly with sulphuric acid and heat to boiling on a water-bath. The presence of bilirubin is indicated by the alcohol assuming a green color.

9. **Bile Acids**.—Extract a small amount of feces with alcohol and filter. Evaporate the filtrate on a water-bath to drive off the alcohol and dissolve the residue in water made slightly alkaline with potassium hydroxide. Upon this aqueous solution try any of the tests for bile acids given on page 122.

10. **Caseinogen**.—Extract the fresh feces first with a dilute solution of sodium chloride, and later with water acidified with dilute acetic acid, to remove soluble proteids. Now extract the feces with 0.5 per cent sodium carbonate and filter. Add dilute acetic acid to the filtrate to precipitate the caseinogen, being careful not to add an excess of the reagent as the caseinogen would dissolve. Filter off the caseinogen and test it according to directions given on page 192. Caseinogen is found principally in the feces of children who have been fed a milk diet. Mucin would also be extracted by the dilute alkali, if present in the feces. What test could you make on the newly precipitated body to differentiate between mucin and caseinogen?

11. **Nucleoproteid**.—Mix the stool thoroughly with water, transfer to a flask, and add an equal amount of saturated lime water. Shake frequently for a few hours, filter, and precipitate the nucleoproteid with acetic acid. Filter off this precipitate and test it as follows:

(a) *Phosphorus*.—Test for phosphorus by fusion (see page 223).

(b) *Solubility*.—Try the solubility in the ordinary solvents.

(c) *Proteid Color Test*.—Try any of the proteid color tests.

What proof have you that the above body was not mucin?

What other test can you use to differentiate between nucleoproteid and mucin?

12. **Albumin and Globulin.**—Extract the fresh feces with a dilute solution of sodium chloride. (The preliminary extract from the preparation of caseinogen, page 146, may be utilized here). Filter, and saturate a portion of the filtrate with sodium chloride in substance. A precipitate signifies globulin. Filter off the precipitate and acidify the filtrate slightly with dilute acetic acid. A precipitate at this point signifies albumin. Make a proteid color test on each of these bodies.

13. **Proteose and Peptone.**—Heat to boiling the portion of the sodium chloride extract not used in the last experiment. Filter off the coagulum, if any forms. Acidify the filtrate slightly with acetic acid and saturate with sodium chloride in substance. A precipitate here indicates proteose. Filter it off and test it according to directions given on page 59. Test the filtrate for peptone by the biuret test.

14. **Inorganic Constituents.**—Prepare a dilute aqueous solution of dry feces and decolorize it by means of purified animal charcoal. Make the following tests upon the clear solution:

(a) *Chlorides.*—Acidify with nitric acid and add silver nitrate.

(b) *Phosphates.*—Acidify with nitric acid, add molybdic solution and warm gently.

(c) *Sulphates.*—Acidify with hydrochloric acid, add barium chloride and warm.

CHAPTER XI.

BLOOD.

Blood is composed of three types of form-elements (erythrocytes or red blood corpuscles, leucocytes or white blood corpuscles and blood plates or plaques) held in suspension in a fluid called *blood plasma*. These form-elements compose about 60 per cent of the blood, by weight. Ordinarily blood is a dark red, opaque fluid due to the presence of the red blood corpuscles, but through the action of certain substances such as water, ether or chloroform it may be rendered transparent. Blood so altered is said to be *laked*. The laking process is simply a liberation of the hæmoglobin from the stroma of the red blood corpuscle. Normal blood is alkaline in reaction¹ to litmus, the alkalinity being due principally to sodium carbonate. The specific gravity of the blood of adults ordinarily varies between 1.045 and 1.075. It varies somewhat with the sex, the blood of males having a rather higher specific gravity than that of females. Under pathological conditions also the density of the blood may be very greatly altered. The freezing-point (Δ) of normal blood is about -0.56° C. Variations between -0.51° and -0.62° C. may be due entirely to dietary conditions, but if any marked variation is noted it can, in most cases, be traced to a disordered kidney function. The total amount of blood in the body has been variously estimated at from one-twelfth to one-fourteenth of the body weight. Perhaps $1/13.5$ is the most satisfactory figure.

Among the most important constituents of blood plasma are the four proteid bodies, *fibrinogen*, *nucleoproteid*, *serum globulin* (euglobulin and pseudo-globulin) and *serum albumin*. Plasma contains about 8.2 per cent of solids of which the

¹ Recently it has been shown by physico-chemical methods that the blood is in reality neutral in reaction.

proteid constituents named above constitute approximately 84 per cent and the inorganic constituents (mainly chlorides, phosphates and carbonates) approximately 10 per cent. Among the inorganic constituents sodium chloride predominates. To prevent coagulation, blood plasma is ordinarily studied in the form of an *oxalated* or *salted* plasma. The former may be obtained by allowing the blood to flow from an opened artery into an equal volume of 0.2 per cent ammonium oxalate solution, whereas in the preparation of a *salted* plasma 10 per cent sodium chloride solution may be used as the diluting fluid.

Fibrinogen is perhaps the most important of the proteid constituents of the plasma. It is also found in lymph and chyle as well as in certain exudates and transudates. Fibrinogen possesses the general properties of the globulins, but differs from serum globulin in being precipitated upon half-saturation with sodium chloride. In the process of coagulation of the blood the fibrinogen is transformed into fibrin. This fibrin is one of the principal constituents of the ordinary blood clot.

The nucleo-proteid of blood possesses many of the characteristics of serum globulin. In common with this body it is easily soluble in sodium chloride, and is completely precipitated from its solutions upon saturation with magnesium sulphate. It is much less soluble in dilute acetic acid than serum globulin and its solutions coagulate at 65°–69° C.

The body formerly called serum globulin is probably not an individual substance. Recent investigations seem to indicate that it may be resolved into two individual bodies called *euglobulin* and *pseudoglobulin*. The euglobulin is practically insoluble in water and may be precipitated in the presence of 28–36 per cent of saturated ammonium sulphate solution. The pseudoglobulin, on the contrary, is soluble in water and is only precipitated by ammonium sulphate in the presence of from 36 to 44 per cent of saturated ammonium sulphate solution.

In common with serum globulin the body known as serum albumin seems also to consist of more than a single individual

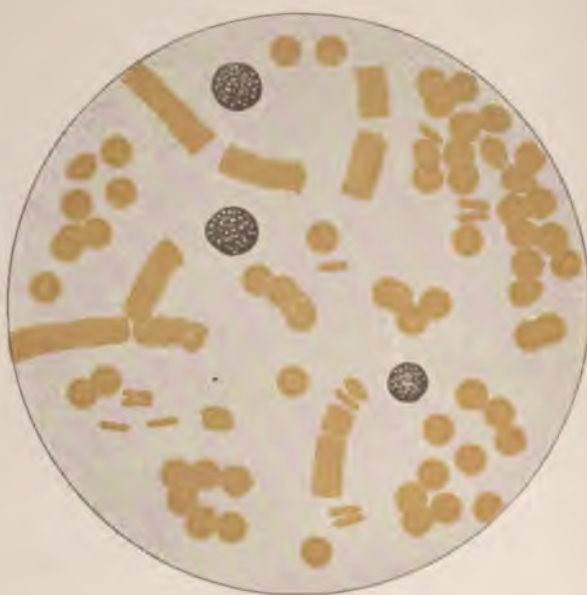
substance. The so-called serum albumin may be separated into at least two distinct bodies, one capable of crystallization, the other an amorphous body. The solution of either of these bodies in water gives the ordinary albumin reactions. The coagulation temperature of the serum albumin mixture as it occurs in serum or plasma varies from 70° to 85° C. according to the reaction of the solution and its content of inorganic material. Serum albumin differs from egg albumin in being more lævorotatory, in being rendered less insoluble by alcohol, and in the fact that when precipitated by hydrochloric acid it is more easily soluble in an excess of the reagent.

When blood coagulates and the usual clot forms, a light yellow fluid exudes. This is blood *serum*. It differs from blood plasma in containing a large amount of *fibrin ferment*, a body of great importance in the coagulation of the blood, and also in possessing a lower proteid content. The proteid material present in plasma and not found in serum is the fibrinogen which is transformed into fibrin in the process of coagulation and removed. The specific gravity of the serum of human blood varies between 1.026 and 1.032.

Beside the proteid constituents already mentioned, other bodies which are found in both the plasma and serum are the following: *Sugar* (dextrose), *fat*, *enzymes*, *lecithin*, *cholesterin* and its esters, *gases*, *coloring-matter* (lutein or lipochrome) and *mineral substances*. In addition to these bodies the following substances have been detected in normal human blood: *Creatin*, *carbamic acid*, *hippuric acid*, *paralactic acid*, *urea* and *uric acid*. Some of the *pathological constituents* of blood are, *proteoses*, *leucin*, *tyrosin* and other amino acids, *biliary constituents* and *purin bodies*.

There has recently been considerable controversy regarding the form of the erythrocytes or red blood corpuscles of human blood. It is claimed by some investigators that the cells are *bell-shaped* or *cup-shaped*. As the erythrocytes occur normally in the circulation, however, they are probably thin, non-nucleated, biconcave discs. When examined singly, under

PLATE IV.



NORMAL ERYTHROCYTES AND LEUCOCYTES.

the microscope, they possess a pale greenish-yellow color (see Plate IV, opposite), whereas when grouped in large masses a reddish tint is noted.

The blood of most mammals contains erythrocytes similar in form to those of human blood. In the blood of a few mammals, however, such as the llama and camel as well as in the blood of birds, fishes, amphibians and reptiles the erythrocytes are ordinarily more or less elliptical, biconvex and possess a nucleus. The erythrocytes vary in size with the different animals. The average diameter of the erythrocytes of blood from various species is given in the following table:¹

Elephant.....	$\frac{37}{1000}$	of an inch.
Guinea-pig.....	$\frac{11}{1000}$	of an inch.
Man.....	$\frac{1250}{1000000}$	of an inch.
Monkey.....	$\frac{1}{3333}$	of an inch.
Dog.....	$\frac{1}{3500}$	of an inch.
Rat.....	$\frac{1}{3666}$	of an inch.
Rabbit.....	$\frac{1}{3555}$	of an inch.
Mouse.....	$\frac{1}{3777}$	of an inch.
Lion.....	$\frac{1}{1143}$	of an inch.
Ox.....	$\frac{1}{1219}$	of an inch.
Horse.....	$\frac{1}{1213}$	of an inch.
Pig.....	$\frac{1}{1368}$	of an inch.
Cat.....	$\frac{1}{1372}$	of an inch.
Sheep.....	$\frac{1}{1611}$	of an inch.
Goat.....	$\frac{1}{1619}$	of an inch.
Musk-deer.....	$\frac{1}{12325}$	of an inch.

The erythrocytes from whatever source obtained, consist essentially of two parts, the *stroma* or protoplasmic tissue and its enclosed pigment, *hæmoglobin*. For human blood the number of erythrocytes present in the fluid as obtained from well-developed males in good physical condition is about 5,500,000 per cubic millimeter.² The normal content of the blood of adult females is from 4,000,000 to 4,500,000 per cubic millimeter. The number of erythrocytes varies greatly

¹ Wormley's Micro-Chemistry of Poisons, second edition, p. 733.

² This statement is based upon observations made upon the blood of athletes in training. It is generally stated in text-books that the blood of males contains about 5,000,000 per cubic millimeter.

FIG. 50.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE GUINEA PIG.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of the University of Pennsylvania.

FIG. 51.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE RAT.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of the University of Pennsylvania.

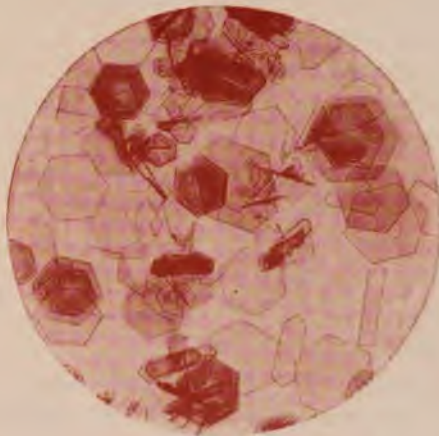
FIG. 52.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE HORSE.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of
the University of Pennsylvania.

FIG. 53.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE SQUIRREL.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of
the University of Pennsylvania.

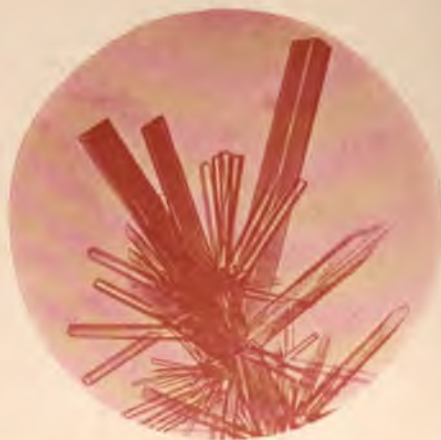
FIG. 54.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE DOG.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of the University of Pennsylvania.

FIG. 55.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE CAT.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of the University of Pennsylvania.

FIG. 56.



OXYHÆMOGLOBIN CRYSTALS FROM BLOOD OF THE NECTURUS.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert of the University of Pennsylvania.¹

under different conditions. For instance the number may be increased after the transfusion of blood of the same species of animal; by residing in a high altitude; or as a result of strenuous physical exercise continued over a short period of time. An increase is also noted in starvation; after partaking of food; after cold or hot baths; after massage, as well as after the administration of certain drugs and accompanying certain diseases such as cholera, diarrhœa, dysentery and yellow atrophy of the liver. A decrease in the number occurs in the different forms of anæmia. The number has been known to increase to 7,040,000 per cubic millimeter as a result of physical exercise, while 11,000,000 per cubic millimeter have been noted in cases of polycythæmia and increases nearly as great in cyanosis. The number has been known to decrease to 500,000 per cubic millimeter or lower in pernicious anæmia.

Oxyhæmoglobin the coloring matter of the blood is a com-

¹The micro-photographs of oxyhæmoglobin (see pages 152-155) and hæmin (see page 164) are reproduced through the courtesy of Professors E. T. Reichert and Amos P. Brown, of the University of Pennsylvania, who are investigating the crystalline forms of biochemic substances.

pound proteid. Through treatment with hydrochloric acid it may be split into a proteid body called *globin*, and *hæmochromogen* an iron-containing pigment. The latter body is rapidly transformed into *hæmatin* in the presence of oxygen and this in turn gives place to hæmatin-hydrochloride or *hæmin* (Figs. 58 and 59, page 164). The pigment of arterial blood is for the most part loosely combined with oxygen and is termed *oxyhæmoglobin*, whereas the pigment of venous blood is principally hæmoglobin (so-called *reduced* hæmoglobin). Oxyhæmoglobin is the oxygen-carrier of the body and belongs to the class of bodies known as respiratory pigments. The reduction of oxyhæmoglobin to form hæmoglobin (so-called *reduced* hæmoglobin) occurs in the capillaries. Oxyhæmoglobin may be crystallized and a specific form of crystal obtained from the blood of each individual species (see Figs. 50 to 56, pages 152 to 155). This fact seems to indicate that there are many varieties of oxyhæmoglobin. The pigment is held within the stroma of the erythrocyte. The following bodies may be derived from hæmoglobin, and each possesses a specific spectrum which serves as an aid in its detection and identification: Oxyhæmoglobin, methæmoglobin, carbon-monoxide hæmoglobin, nitric-oxide hæmoglobin, hæmochromogen, hæmatin, acid-hæmatin, alkali-hæmatin and hæmatoporphyrin (see Absorption Spectra, Plates I and II).

The white corpuscles (or leucocytes) of human blood differ from the red corpuscles (or erythrocytes) in being somewhat larger in size, in containing at least a single nucleus and in possessing amœboid movement (see Plate IV, opposite page 151). They are typical animal cells and therefore contain the following bodies which are customarily present in such cells: *Proteids, fats, carbohydrates, lecithin, cholesterolin, inorganic salts and water*. The normal number of leucocytes in human blood varies between 5,000 and 10,000 per cubic millimeter. The ratio between the leucocytes and erythrocytes is about 1:350-500. A *leucocytosis* is said to exist when the number of leucocytes is increased for any reason. Leucocytoses may

be divided into two general classes, the *physiological* and the *pathological*. Under the physiological form would be classed those leucocytoses accompanying pregnancy, parturition and digestion, as well as those due to mechanical and thermal influences. The leucocytoses spoken of as pathological are the inflammatory, infectious, post-hæmorrhagic, toxic and experimental forms as well as the type of leucocytosis which accompanies malignant disease.

The blood plates (platelets or plaques) are round or oval, colorless discs which possess a diameter about one-third as great as that of the erythrocytes. Upon treatment with certain reagents, *e. g.*, artificial gastric juice, they may be separated into a homogeneous, non-refractive portion and a granular, refractive portion. The blood plates are probably associated in some way with the coagulation of the blood. This relationship is not well understood at present.

The processes involved in the coagulation of the blood are not fully understood. Several theories have been advanced and each has its adherents. The theory which appears to be fully as firmly founded upon experimental evidence as any is the following: Blood contains a zymogen called *prothrombin* which combines with the calcium salts present to form an enzyme known as *thrombin* or *fibrin-ferment*. When freshly drawn blood comes in contact with the air the fibrin-ferment at once acts upon the fibrinogen present and gives rise to the formation of *fibrin*. This fibrin forms in shreds throughout the blood mass and, holding the form elements of the blood within its meshes, serves to produce the typical *blood clot*. The fibrin shreds gradually contract, the whole clot assumes a jelly-like appearance and the yellowish serum exudes. If, immediately upon the withdrawal of blood from the body, the fluid be rapidly stirred or thoroughly "whipped" with a bundle of coarse strings, twigs or a specially constructed beater, the fibrin shreds will not form in a network throughout the blood mass but instead will cling to the device used in beating. In this way the fibrin may be removed and the

remaining fluid is termed *defibrinated* blood. The above theory of the coagulation of the blood may be stated briefly as follows:

I. Prothrombin + Calcium Salts = Thrombin (or Fibrin-ferment).

II. Thrombin (or Fibrin-ferment) + Fibrinogen = Fibrin.

Among the medico-legal tests for blood are the following: (1) Microscopical identification of the erythrocytes, (2) spectroscopic identification of blood solutions, (3) the guaiac test, (4) preparation of hæmin crystals. Of these four tests the last named is generally considered to be the most satisfactory. It gives equally reliable results with fresh blood and with blood from clots or stains of long standing, provided the latter have not been exposed to a high temperature, or to the rays of the sun for a long period. The technique of the test is simple (see page 163) and the formation of the dark brown or chocolate colored crystals of hæmin (Figs. 58 and 59, page 164) is indisputable proof of the presence of blood in the fluid, clot or stain examined. The weak point of the test, medico-legally, lies in the fact that it does not differentiate between human blood and that of certain other species of animal.

The guaiac test (see page 163), although generally considered less accurate than the hæmin test, is really a more delicate test than the hæmin test if properly performed. One of the most common mistakes in the manipulation of this test is the use of a guaiac solution which is too concentrated and which, when brought into contact with the aqueous blood solution, causes the separation of a voluminous precipitate of a resinous material which may obscure the blue coloration; this is particularly true of the test when used for the examination of blood stains. A solution of guaiac made by dissolving 1 gram of the resin in 60 c.c. of 95 per cent alcohol is very satisfactory for general use. The test is frequently objected to upon the ground that various other substances, *e. g.*, milk, pus, saliva, etc., respond to the test and that it cannot therefore

be considered a specific test for blood and is of value only in a negative sense. We have demonstrated to our own satisfaction, however, that milk many times gives the blue color upon the addition of an alcoholic solution of guaiac resin without the addition of hydrogen peroxide or old turpentine. Buckmaster has very recently advocated the use of an alcoholic solution of guaiaconic acid instead of an alcoholic solution of guaiac resin. He claims that he was able to produce the blue color upon the addition of the guaiaconic acid to milk *only* when the sample of milk tested was brought from the country in *sterile bottles*, and further, that no sample of London milk which he examined responded to the test. In the application of the guaiac test to the detection of blood, he states that he was able to detect *laked blood* when present in the ratio 1:5,000,000 and *unlaked blood* when present in the ratio 1:1,000,000. This author considers the guaiac test to be far more trustworthy than is generally believed.

Up to within very recent times it has been impossible to make an absolute differentiation of human blood. Recently however the so-called "biological" blood test has made such a differentiation possible. This test, known as the Bordet reaction, is founded upon the fact that the blood serum of an animal into which has been injected the blood of another animal of different species develops the property of agglutinating and dissolving erythrocytes *similar to those injected*, but exerts this influence upon blood from *no other species*. The antiserum used in this test is prepared by injecting rabbits with 5-10 c.c. of human defibrinated blood, at intervals of about four days until a total of between 50 and 80 c.c. has been injected. After a lapse of one or two weeks the animal is bled, the serum collected, placed in sterile tubes and preserved for use as needed. In examining any specific solution for human blood it is simply necessary to combine the antiserum and the solution under examination in the proportion of 1:100 and place the mixture at 37° C. If human blood is present in the solution a turbidity will be noted and this will change

within three hours to a distinctly flocculent precipitate. This antiserum will react thus with no other known substance.

EXPERIMENTS ON BLOOD.

I. Defibrinated Ox-blood.

1. **Reaction.**—Moisten red and blue litmus papers with 10 per cent sodium chloride solution and test the reaction of the defibrinated blood.

2. **Microscopical Examination.**—Examine a drop of defibrinated blood under the microscope. Compare the objects you observe with Plate IV, opposite page 151. Repeat the test with a drop of your own blood.

3. **Specific Gravity.**—Determine the specific gravity of defibrinated blood by means of an ordinary specific gravity spindle. Compare this result with the specific gravity as determined by Hammerschlag's method in the next experiment.

4. **Specific Gravity by Hammerschlag's Method.**—Fill an ordinary urinometer cylinder about one-half full of a mixture of chloroform and benzene, having a specific gravity of approximately 1.050. Into this mixture allow a drop of the blood under examination to fall from a pipette or directly from the finger in case fresh blood is being examined. Care must be taken not to use too large a drop of blood and to keep the drop from coming in contact with the walls of the cylinder. If the blood drop sinks to the bottom of the vessel, thus showing it to be of higher specific gravity than the surrounding fluid, add chloroform until the blood drop remains suspended in the mixture. Stir carefully with a glass rod after adding the chloroform. If the blood drop rises to the surface upon being introduced into the mixture, thus showing it to be of lower specific gravity than the surrounding fluid, add benzene until the blood drop remains suspended in the mixture. Stir with a glass rod after the benzene is added. After the blood drop has been brought to a suspended position in the mixture by means of one or more additions of chloroform and benzene this final mixture should be filtered through muslin and its

specific gravity accurately determined. What is the specific gravity of the blood under examination?

5. **Tests for Various Constituents.**—Place 10 c.c. of defibrinated blood in an evaporating dish, dilute with 100 c.c. of water and heat to boiling. Is there any coagulation, and if so what bodies form the coagulum? At the boiling-point acidulate slightly with dilute acetic acid. Filter. The filtrate should be clear and the coagulum dark brown. Reserve this coagulum. What body gives the coagulum this color? Evaporate the filtrate to about 25 c.c., filtering off any precipitate which may form in the process. Make the following tests upon the filtrate:

(a) *Fehling's Test.*—Test for sugar according to directions given on page 8.

(b) *Chlorides.*—To a small amount of the filtrate in a test-tube add a few drops of nitric acid and a little argentic nitrate. In the presence of chlorides, a white precipitate of argentic chloride will form.

(c) *Phosphates.*—Test for phosphates by nitric acid and molybdic solution according to directions given on page 37.

(d) *Proteose and Peptone.*—Test a small amount of the solution for proteose and peptone by saturating with ammonium sulphate according to directions given on page 59.

(e) *Crystallization of Sodium Chloride.*—Place the remainder of the filtrate in a watch glass and evaporate it on a water-bath. Examine the crystals under the microscope and compare them with those in Fig. 60, page 167.

6. **Test for Iron.**—Incinerate a small portion of the coagulum from the last experiment (5) in a porcelain crucible. Cool, dissolve the residue in dilute hydrochloric acid and test for iron by potassium ferrocyanide or ammonium sulphocyanide. Which of the constituents of the blood contains the iron?

7. **Laky Blood.**—Note the opacity of ordinary defibrinated blood. Place a few cubic centimeters of this blood in a test-tube and add water, a little at a time, until the blood is rendered transparent. It is now *laky* blood. How does the water

act in causing this transparency? Examine a drop of laky blood under the microscope. How does its microscopical appearance differ from that of unaltered blood? What other agents may be used to render blood laky?

FIG. 57.



EFFECT OF WATER ON ERYTHROCYTES.

8. **Osmotic Pressure.**—Place a few cubic centimeters of blood in each of three test-tubes. Take the blood in the first tube according to directions given in the last experiment (7): add an equal volume of *isotonic* (0.9 per cent) sodium chloride to the blood in the second tube, and an equal volume of 10 per cent sodium chloride to the blood in the third tube. Mix thoroughly by shaking and after a few moments examine a drop from each of the three tubes under the microscope (see Figs. 57 and 115, pages 162 and 337). What do you find and what is your explanation from the standpoint of osmotic pressure?

9. **Diffusion of Hæmoglobin.**—Prepare some laky blood, thus liberating the hæmoglobin from the erythrocytes. Test the diffusion of the hæmoglobin by preparing a dialyzer like one of the models shown in Fig. 1, page 6. How does hæmoglobin differ from other well-known crystallizable bodies?

10. **Guaiac Test.**—To 5 c.c. of water in a test-tube add two drops of blood. By means of a pipette drop an alcoholic solution of guaiac (strength about 1:60)¹ into the resulting mixture until a turbidity is observed and add old turpentine or hydrogen peroxide, drop by drop, until a blue color is obtained. Do any other substances respond in a similar manner to this test? Is a positive guaiac test a sure indication of the presence of blood?

11. **Hæmin Test.**—(a) *Teichmann's Method.*—Place a *very small* drop of blood on a microscopic slide, add a minute grain of sodium chloride² and *carefully* evaporate to *dryness* over a *low flame*. Put a cover glass in place, run underneath it a drop of *glacial* acetic acid and *warm gently* until the formation of gas bubbles is noted. Cool the preparation, examine under the microscope and compare the crystals with those shown in Figs. 58 and 59, page 164. The hæmin crystals result from the decomposition of the hæmoglobin of the blood. What are the steps involved in this process? The hæmin crystals are also called Teichmann's crystals. Is this an *absolute* test for blood? Is it possible to differentiate between human blood and the blood of other species by means of the hæmin test?

(b) *Zeynek and Nencki's Method.*—To 10 c.c. of defibrinated blood add acetone until no more precipitate forms. Filter off the precipitated proteid and extract it with 10 c.c. of acetone made acid with 2–3 drops of hydrochloric acid. Place a drop of the resulting colored extract on a slide, immediately place a cover glass in position and examine under the microscope. Upon the evaporation of the acetone, crystals of hæmin will form. Larger crystals may be obtained by evaporating the acetone extract about one-half, transferring it to a stoppered vessel and allowing it to remain over night.

(c) *Schalfjew's Method.*—Place 20 c.c. of glacial acetic

¹ Buckmaster advises the use of an alcoholic solution of guaiacetic acid instead of an alcoholic solution of guaiac resin.

² Buckmaster considers the use of potassium chloride preferable.

FIG. 58.



HÆMIN CRYSTALS FROM HUMAN BLOOD.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert, of the University of Pennsylvania.

FIG. 59.



HÆMIN CRYSTALS FROM SHEEP BLOOD.

Reproduced from a micro-photograph furnished by Prof. E. T. Reichert, of the University of Pennsylvania.

acid in a small beaker and heat to 80° C. Add 5 c.c. of strained defibrinated blood, again bring the temperature to 80° C., remove the flame and allow the mixture to cool. Examine the crystals under the microscope and compare them with those reproduced in Figs. 58 and 59, page 164.

12. **Catalytic Action.**—To about 10 drops of blood in a test-tube add twice the volume of hydrogen peroxide, without shaking. The mixture foams. What is the cause of this phenomenon?

13. **Preparation of Hæmatin.**—Place 100 c.c. of *laked* blood in a beaker and add 95 per cent alcohol until precipitation ceases. What bodies are precipitated? Transfer the precipitate to a flask and boil with 95 per cent alcohol previously acidulated with sulphuric acid. Through the action of the acid the hæmoglobin is split into hæmatin and a proteid body called globin. Later the "sulphuric acid ester of hæmatin" is formed, which is soluble in the alcohol. Continue heating until the precipitate is no longer colored, then filter. Partly saturate the filtrate with sodium chloride and warm. In this process the "hydrochloric acid ester of hæmatin" is formed. Filter and dissolve on the filter paper by sodium carbonate. Save this alkaline solution of hæmatin and make a spectroscopic examination later after becoming familiar with the use of the spectroscope. How does the spectrum of oxyhæmoglobin differ from that of the derived *alkali hæmatin*?

14. **Variation in Size of Erythrocytes.**—Prepare two small funnels with filter papers such as are used in quantitative analysis. Moisten each paper with normal (isotonic) salt solution. Into one funnel introduce a small amount of defibrinated ox blood and into the other funnel allow blood to drop directly from a decapitated frog. Note that the filtrate from the ox blood is colored whereas that from the frog blood is colorless. What deduction do you make regarding the relative size of the erythrocytes in ox and frog blood? Does either filtrate clot? Why?

II. Blood Serum.

1. **Coagulation Temperature.**—Place 5 c.c. of undiluted serum in a test-tube and determine its temperature of coagulation according to the method described on page 50. Note the temperature at which a cloudiness occurs as well as the temperature at which coagulation is complete.

2. **Precipitation by Alcohol.**—To 5 c.c. of serum in a test-tube add twice the amount of 95 per cent alcohol and thoroughly mix by shaking. What is this precipitate? Make a confirmatory test. Test the alcoholic filtrate for proteid. Explain the result.

3. **Proteids of Blood Serum.**—Place about 20 c.c. of undiluted serum in a small evaporating dish, heat to boiling and at the boiling-point acidify slightly with dilute acetic acid. Of what does this coagulum consist? Filter off the coagulum (reserve the filtrate) and test it as follows:

(a) *Millon's Reaction.*—Make the test according to directions given on page 44.

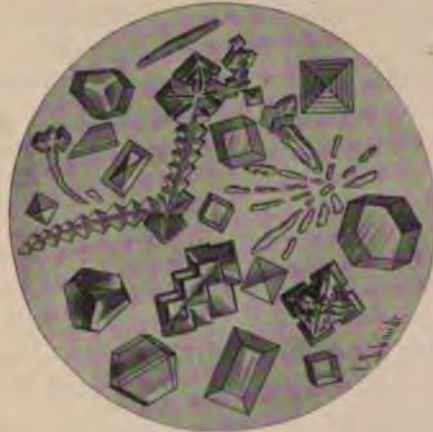
(b) *Xanthoproteic Test.*—Make the test according to directions given on page 44.

4. **Sugar in Serum.**—Test a little of the filtrate from Experiment 3 by Fehling's test. What do you conclude?

5. **Detection of Sodium Chloride.**—(a) Test a little of the filtrate from Experiment 3 for chlorides, by the use of nitric acid and argentic nitrate. (b) Evaporate 5 c.c. of the filtrate from Experiment 3 in a watch glass on a water-bath. Examine the crystals and compare them with those reproduced in Fig. 60, page 167.

6. **Separation of Serum Globulin and Serum Albumin.**—Place 10 c.c. of blood serum in a small beaker and saturate with magnesium sulphate. What is this precipitate? Filter it off and acidify the filtrate slightly with acetic acid. What is this second precipitate? Filter this precipitate off and test the filtrate by the biuret test. What do you conclude?

FIG. 60.



SODIUM CHLORIDE.

III. Blood Plasma.

1. **Preparation of Oxalated Plasma.**—Allow arterial blood to run into an equal volume of 0.2 per cent ammonium oxalate solution.

2. **Preparation of Fibrinogen.**—To 25 c.c. of oxalated plasma add an equal volume of saturated sodium chloride solution. Note the precipitation of fibrinogen. Filter off the precipitate (reserve the filtrate) and test it by a proteid color test (see page 44).

3. **Effect of Calcium.**—Place a small amount of oxalated plasma in a test-tube and add a few drops of a 2 per cent calcium chloride solution. What occurs? Explain it.

4. **Preparation of Salted Plasma.**—Allow arterial blood to run into an equal volume of a saturated solution of sodium sulphate or a 10 per cent solution of sodium chloride. Keep the mixture in a cold place for about twenty-four hours.

5. **Effect of Dilution.**—Place a few drops of salted plasma in a test-tube and dilute it with 10–15 volumes of water. What do you observe? Explain it.

6. **Crystallization of Oxyhæmoglobin.**—*Reichert's Method.*—Allow the blood of the dog or horse to flow into an equal

volume of 7 per cent ammonium oxalate solution. Place a small amount of this oxalated blood in a test-tube and lake it with ether, being careful to avoid an excess of the reagent. By means of a pipette transfer a drop of this laked blood to a slide, and when the edges of the drop begin to dry place a cover glass in position. Examine under the microscope and compare the crystals with those in Figs. 50 to 56, pages 152 to 155.

IV. Fibrin.

1. **Preparation of Fibrin.**—Allow blood to flow directly from the animal into a vessel and rapidly *whip* it by means of a bundle of twigs, a mass of strong cords or a specially constructed beater. If a pure fibrin is desired it is not best to attempt to manipulate a large volume of blood at one time. After the fibrin has been collected it should be freed from any adhering blood clots and washed in water to remove further traces of blood. The pure product should be very light in color. It may be preserved under glycerin, dilute alcohol or chloroform water.

2. **Solubility.**—Try the solubility of small shreds of freshly prepared fibrin in the usual solvents.

3. **Millon's Reaction.**—Make the test according to directions given on page 44.

4. **Xanthoproteic Test.**—Make the test according to directions given on page 44.

5. **Biuret Test.**—Make the test according to directions given on page 45.

V. Detection of Blood in Stains on Cloth, etc.

1. **Identification of Corpuscles.**—If the stain under examination is on cloth a portion should be extracted with a few drops of glycerin or normal (0.9 per cent) sodium chloride solution. A drop of this solution should then be examined under the microscope to determine if corpuscles are present.

2. **Tests on Aqueous Extract.**—A second portion of the stain should be extracted with a small amount of water and the following tests made upon the aqueous extract:

(a) *Hæmochromogen*.—Make a small amount of the extract alkaline by potassium hydroxide or sodium hydroxide, and heat until a brownish-green color results. Cool and add a few drops of ammonium sulphide or Stokes' reagent (see page 170) and make a spectroscopic examination. Compare the spectrum with that of hæmochromogen (see Absorption Spectra, Plate II).

(b) *Hæmin Test*.—Make this test upon a small drop of the aqueous extract according to the directions given on page 163.

(c) *Guaiac Test*.—Make this test on the aqueous extract according to the directions given on page 163. The guaiac solution may also be applied directly to the stain without previous extraction in the following manner: Moisten the stain with water, and after allowing it to stand several minutes, add an alcoholic solution of guaiac (strength about 1:60) and a little hydrogen peroxide or old turpentine. The customary blue color will be observed in the presence of blood.

(d) *Acid Hæmatin*.—If the stain fails to dissolve in water extract with acid alcohol and examine the spectrum for absorption bands of acid hæmatin (see Absorption Spectra, Plate II).

VI. Spectroscopic Examination of Blood.

(For Absorption Spectra see Plates I. and II.).

Either the *angular*-vision spectroscope (Figs. 62 and 63, pages 170 and 171) or the *direct*-vision spectroscope (Fig. 61, page 170) may be used in making the spectroscopic examination of the blood. For a complete description of these instruments the student is referred to any standard text-book of physics.

1. *Oxyhæmoglobin*.—Examine dilute (1:50) defibrinated blood spectroscopically. Note the broad absorption-band between D and E. Continue the dilution until this single broad band gives place to two narrow bands, the one nearer the D line being the narrower. These are the typical absorption-bands of oxyhæmoglobin obtained from dilute solutions of

blood. Now dilute the blood *very freely* and note that the bands gradually become more narrow and, if the dilution is sufficiently great, they finally entirely disappear.

FIG. 61.



DIRECT-VISION SPECTROSCOPE.

2. Hæmoglobin (so-called *Reduced Hæmoglobin*).—To blood which has been diluted sufficiently to show well defined oxyhæmoglobin absorption-bands add a small amount of

FIG. 62.



ANGULAR-VISION SPECTROSCOPE ARRANGED FOR ABSORPTION ANALYSIS.

Stokes' reagent.¹ The blood immediately changes in color from a bright red to a violet-red. The oxyhæmoglobin has been reduced through the action of Stokes' reagent and

¹ Stokes' reagent is a solution containing 2 per cent ferrous sulphate and 3 per cent tartaric acid. When needed for use a small amount should be placed in a test-tube and ammonium hydroxide added until the precipitate which forms on the first addition of the hydroxide has entirely dissolved. This produces *ammonium ferrotartrate* which is a reducing agent.

hæmoglobin (so-called *reduced* hæmoglobin) has been formed. This has been brought about by the removal of some of the loosely combined oxygen from the oxyhæmoglobin. Examine this hæmoglobin spectroscopically. Note that in place of the two absorption bands of oxyhæmoglobin we now have a single broad band lying almost entirely between D and E. This is

FIG. 63.

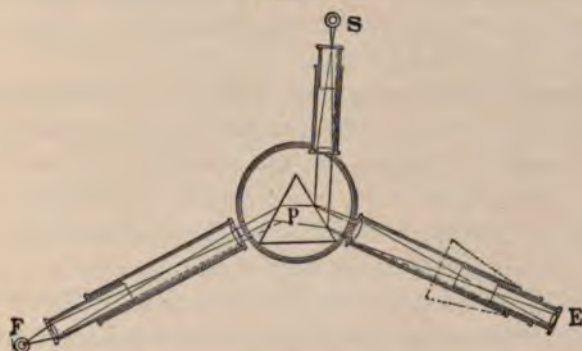


DIAGRAM OF ANGULAR-VISION SPECTROSCOPE. (Long.)

The white light *F* enters the collimator tube through a narrow slit and passes to the prism *P*, which has the power of refracting and dispersing the light. The rays then pass to the double convex lens of the ocular tube and are deflected to the eyepiece *E*. The dotted lines show the magnified virtual image which is formed. The third tube contains a scale whose image is reflected into the ocular and shown with the spectrum. Between the light *F*, and the collimator slit is placed a cell to hold the solution undergoing examination.

the typical spectrum of hæmoglobin. If the solution showing this spectrum be shaken in the air for a few moments it will again assume the bright red color of oxyhæmoglobin and show the characteristic spectrum of that pigment.

3. **Carbon Monoxide Hæmoglobin.**—The preparation of this pigment may be easily accomplished by passing ordinary illuminating gas¹ through defibrinated ox-blood. Blood thus treated assumes a brighter tint (carmine) than that imparted by oxyhæmoglobin. In very dilute solution oxyhæmoglobin

¹ The so-called water gas with which ordinary illuminating gas is diluted contains usually as much as 20 per cent of carbon monoxide (CO).

appears yellowish-red whereas carbon monoxide hæmoglobin under the same conditions appears bluish-red. Examine the carbon monoxide hæmoglobin solution spectroscopically. Observe that the spectrum of this body resembles the spectrum of oxyhæmoglobin in showing two absorption-bands between D and E. The bands of carbon monoxide hæmoglobin, however, are somewhat nearer the violet end of the spectrum. Add some Stokes' reagent to the solution and again examine spectroscopically. Note that the position and intensity of the absorption bands remain unaltered.

4. **Neutral Methæmoglobin.**—Dilute a little defibrinated blood (1:10) and add a few drops of a freshly prepared 10 per cent solution of potassium ferricyanide. Shake this mixture and observe that the bright red color of the blood is displaced by a brownish-red. Now dilute a little of this solution and examine it spectroscopically. Note the single, very dark absorption-band lying to the left of D and, if the dilution is sufficiently great, also observe the two rather faint bands lying between D and E in somewhat similar positions to those occupied by the absorption-bands of oxyhæmoglobin. Add a few drops of Stokes' reagent to the methæmoglobin solution while it is in position before the spectroscope and note the immediate appearance of the oxyhæmoglobin spectrum which is quickly followed by that of hæmoglobin.

5. **Alkaline Methæmoglobin.**—Render a neutral solution of methæmoglobin, such as that used in the last experiment (4), slightly alkaline with a few drops of ammonia. The solution becomes redder in color, due to the formation of alkaline methæmoglobin and shows a spectrum different from that of the neutral body. In this case we have a band on either side of D, the one nearer the red end of the spectrum being much the fainter. A third band, darker than either of those mentioned lies between D and E somewhat nearer E.

6. **Alkali Hæmatin.**—Observe the spectrum of the alkali hæmatin prepared in Experiment 13 on page 165. Also make a spectroscopic examination of a freshly prepared alkali

hæmatin.¹ The typical spectrum of alkali hæmatin shows a single absorption-band lying across D and mainly toward the red end of the spectrum.

7. **Reduced Alkali Hæmatin or Hæmochromogen.**—Dilute the alkali hæmatin solution used in the last experiment (6) to such an extent that it shows no absorption-band. Now add a few drops of Stokes' reagent and note that the greenish-brown color of the alkali hæmatin solution is displaced by a bright red color. This is due to the formation of hæmochromogen or reduced alkali hæmatin. Examine this solution spectroscopically and observe the narrow, dark, absorption-band lying midway between D and E. If the dilution is not too great a faint band may be observed in the green extending across E and b. ary

8. **Acid Hæmatin.**—To some defibrinated blood add half its volume of glacial acetic acid and an equal volume of ether. Mix thoroughly. The acidified ethereal solution of hæmatin rises to the top and may be poured off and used for the spectroscopic examination. If desired it may be diluted with acidified ether in the ratio of one part of glacial acetic acid to two parts of ether. A distinct absorption-band will be noted in the red between C and D and lying somewhat nearer C than the band in the methæmoglobin spectrum. Between D and F may be seen a rather indistinct broad band. Dilute the solution until this band resolves itself into two bands. Of these the more prominent is a broad, dark absorption-band lying in the green between b and F. The second, a narrow band of faint outline, lies in the light green to the red side of E. A fourth very faint band may be observed lying on the violet side of D.

9. **Acid Hæmatoporphyrin.**—To 5 c.c. of concentrated sulphuric acid in a test-tube add two drops of blood mixing

¹ Alkali hæmatin may be prepared by mixing one volume of a concentrated potassium hydroxide or sodium hydroxide solution and two volumes of dilute (1:5) defibrinated blood. This mixture should be heated gradually almost to boiling, then cooled and shaken for a few moments in the air before examination.

thoroughly by agitation after the addition of each drop. A wine-red solution is produced. Examine this solution spectroscopically. Acid hæmatoporphyrin gives a spectrum with an absorption-band on either side of D, the one nearer the red end of the spectrum being the narrower.

10. **Alkaline Hæmatoporphyrin.**—Introduce the acid hæmatoporphyrin solution just examined into an excess of distilled water. Cool the solution and add potassium hydroxide slowly until the reaction is but slightly acid. A colored precipitate forms which includes the principal portion of the hæmatoporphyrin. The presence of sodium acetate facilitates the formation of this precipitate. Filter off the precipitate and dissolve it in a small amount of dilute potassium hydroxide. Alkaline hæmatoporphyrin prepared in this way forms a bright red solution and possesses four absorption-bands. The first is a very faint, narrow band in the red, midway between C and D; the second is a broader, darker band lying across D, principally to the violet side. The third absorption-band lies principally between D and E, extending for a short distance across E to the violet side, and the fourth band is broad and dark and lies between b and F. The first band mentioned is the faintest of the four and is the first to disappear when the solution is diluted.

VII. Instruments Used in the Clinical Examination of the Blood.

1. **Fleischl's Hæmometer** (Fig. 64, p. 175).—This is an instrument used quite extensively clinically, for the quantitative determination of hæmoglobin. The instrument consists of a small cylinder which is provided with a fixed glass bottom and a movable glass cover, and which is divided, by means of a metal septum, into two compartments of equal capacity. This cylinder is supported in a vertical position by means of a mechanism which resembles the base and stage of an ordinary microscope. Underneath the stage is placed a colored glass wedge (see Fig. 66, p. 176), so arranged as to run immediately beneath the glass bottom of one of the compartments of the

cylinder and ground in such a manner that each part of the wedge corresponds in color to a solution of hæmoglobin of some definite percentage. The glass wedge is held in a metal frame and may be moved backward or forward by means of a rack and pinion arrangement. A scale along the side of this frame indicates the percentage of the normal amount of hæmoglobin which each particular variation in the depth of color of the ground wedge represents, taking the normal hæmoglobin content as 100.¹ In a position corresponding to the position of the mirror on the ordinary microscope is attached a light-colored opaque plate which serves to reflect the light upward through the colored wedge and the cylinder to the eye of the observer.

In making a determination of the percentage of hæmoglobin by this instrument the procedure is as follows: Fill each compartment about three-fourths full of distilled water. Punc-

ture the finger-tip or lobe of the ear of the subject by means of a sterile needle or scalpel and, as soon as a drop of blood appears, place one end of the capillary pipette (Fig. 65), which accompanies the instrument, against the drop and allow it to fill by capillary attraction. To prevent the blood from adhering to the exterior of the tube, and so render the determination inaccurate, it is customary to apply a very thin coating of mutton fat to the outer surface before using or to

FIG. 64.

FLEISCHL'S HÆMOMETER.
(Da Costa.)

FIG. 65.

PIPETTE OF FLEISCHL'S
HÆMOMETER.

¹ The scale of the ordinary instrument is usually too high.

wrap the tube in a piece of oily chamois when not in use. As soon as the tube has been accurately filled with blood it should be dipped into the water of one of the compartments of the cylinder and all traces of the blood washed out with water by means of a small dropper which accompanies the instrument.

FIG. 66.



COLORING GLASS WEDGE OF FLEISCHL'S
HEMOMETER. (*Da Costa*.)

If the blood is not well distributed throughout the compartment and does not form a homogeneous solution the contents of the compartment should be mixed thoroughly by means of the metal handle of the capillary measuring pipette.

When this has been done each compartment should be completely filled with distilled water and the glass cover adjusted, care being taken that the contents of the two compartments do not mix. Now adjust the cylinder so that the compartment containing the pure distilled water is immediately above the colored glass wedge. By means of the rack and pinion arrangement, manipulate the colored wedge, until a portion of it is found which corresponds in color with the diluted blood. When this agreement in color has been secured the point on the scale corresponding to this particular color should be read and the actual percentage of hæmoglobin computed. For instance, if the scale reading is 90 it means that the blood under examination contains 90 per cent of the normal quantity of hæmoglobin, *i. e.*, 90 per cent of 14 per cent.

2. Fleischl-Miescher Hæmometer.—The apparatus of Fleischl has recently been modified by Miescher. If all precautions are taken, the margin of error in the absolute quantity of hæmoglobin determined by this instrument does not exceed 0.15–0.22 per cent by weight of the blood. Detailed directions for the manipulation of the Fleischl-Miescher hæmometer accompany the instrument. In brief Miescher modified the instrument as follows: (1) The scale of each

instrument is supplied with a caliber table of *absolute* hæmoglobin values, expressed in milligrams: the scale of Fleischl's hæmometer shows the percentage of hæmoglobin in relation to an average selected somewhat arbitrarily. Thus many errors arising from the irregular coloring of the glass wedge of the older apparatus are avoided in the instrument as modified. (2) Each instrument is accompanied by a measuring pipette (*melangeur*) which allows of a more accurate measurement of the blood than was possible with the capillary tubes of the older apparatus. (3) With the aid of the measuring pipette mentioned above blood of varying degrees of concentration may be compared. In this way the individual examinations are controlled and a check upon the accuracy of the graduation in the color of the glass wedge is also afforded. This wedge is much more evenly and accurately colored than in the unmodified apparatus of Fleischl. (4) Before reading the percentage as indicated by the scale, the chamber is covered with a glass and a diaphragm which sharply define the field on all sides without the formation of a meniscus.

The measuring pipette is constructed essentially the same as the pipettes which accompany the Thoma-Zeiss Apparatus (see p. 182). The capillary portion, however, is graduated 1, $\frac{2}{3}$ and $\frac{1}{2}$ which enables the observer to dilute the blood sample in the proportion of 1:200, 1:300 or 1:400 as he may desire. If there is difficulty in drawing in the blood exactly to one of the graduations just mentioned the amount of blood above or below the volume indicated by the graduation may be determined by means of certain delicate cross-lines which are placed directly above and below the graduation. Each cross-line corresponds to $\frac{1}{100}$ of the volume of the capillary tube from the tip to the 1 graduation.

A 0.1 per cent solution of sodium carbonate is used to dissolve the stroma of the erythrocytes and so render the blood solution perfectly clear. If this is not done the color of the blood solution invariably appears darker in tone than that of the colored glass wedge. A freshly prepared sodium carbo-

nate solution should be used in order that the clearness of the solution may not be marred by the presence of sodium bicarbonate.

3. **Dare's Hæmoglobinometer** (Fig. 67, below).—This instrument, as the name signifies, is used for the determination of hæmoglobin. In

FIG. 67.



DARE'S HÆMOGLOBINOMETER. (Da Costa.)

R, Milled wheel acting by a friction bearing on the rim of the color disc; S, case inclosing color disc, and provided with a stage to which the blood chamber is fitted; T, movable wing which is swung outward during the observation, to serve as a screen for the observer's eyes, and which acts as a cover to inclose the color disc when the instrument is not in use; U, telescoping camera tube, in position for examination; V, aperture admitting light for illumination of the color disc; X, capillary blood chamber adjusted to stage of instrument, the slip of opaque glass, W, being nearest to the source of light; Y, detachable candle-holder; Z, rectangular slot through which the hæmoglobin scale indicated on the rim of the color disc is read.

(5) a small telescope used in the examination of the areas illuminated by the candle flame, (6) a scale graduated in percentages of the normal amount of hæmoglobin, (7) a hard rubber case, (8) a movable screen attached to the case.

The capillary observation cell is formed of two small,

using either Fleischl's hæmometer or the instrument as modified by Miescher the blood is diluted for examination whereas with the Dare instrument *no dilution* is required. This probably allows of rather more accurate determinations than are possible with the old Fleischl apparatus.

The instrument consists essentially of the following parts: (1) A capillary observation cell, (2) a semicircular colored glass wedge, (3) a milled wheel for manipulating the wedge, (4) a candle used to illuminate portions of the capillary observation cell and the colored wedge,

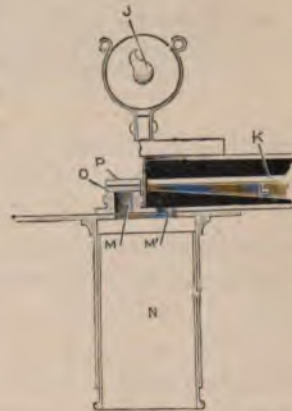
polished rectangular plates of glass, one being transparent and the other opaque. When held in position on the instrument, by means of a small metal bracket, the opaque portion of the cell is nearer the candle and thus serves to soften the glare of light when an observation is being made. The transparent portion of the cell is directly over a circular opening in the case, through which the blood specimen is viewed by means of the small telescope.

The semicircular colored glass wedge is so ground that each particular shade of color corresponds to that possessed by fresh blood which contains some definite percentage of hæmoglobin. It is mounted upon a disc which may be manipulated by the milled wheel in such a manner as to bring successive portions of the wedge in position to be viewed through a circular opening contiguous to the opening through which the blood specimen is viewed. For a further description of the instrument see Figures 67, 68 and 69, on pages 178, 179, and 180 respectively.

In using the Dare hæmoglobi-nometer proceed as follows: Puncture the finger-tip or lobe of the ear of the subject by means of a needle or scalpel and, after a drop of blood

of good proportions has formed, place the flat capillary observation cell in contact with the drop and allow it to fill by capillary attraction (Fig. 69, page 180). Replace the cell in its proper place on the instrument. When in position, a portion of this cell may be observed through a small telescope attached to the apparatus. It is viewed through a circular opening and near this circle is a second one through which a portion of a semi-circular colored glass wedge is visible. These two circles are

FIG. 68.



HORIZONTAL SECTION OF DARE'S
HÆMOGLOBINOMETER.
(Da Costa.)

illuminated simultaneously by means of the flame of a candle. The colored glass may be rotated by means of a milled wheel and the point of agreement of the color of the adjoining discs may be determined in the same way as in Fleischl's hæmometer. The scale reading gives the percentage of the normal

FIG. 69.



METHOD OF FILLING THE CAPILLARY OBSERVATION CELL OF DARE'S HÆMOGLOBINOMETER. (*Da Costa.*)

quantity of hæmoglobin which the blood sample under examination contains. Compute the actual hæmoglobin content in the same manner as from the scale reading of the Fleischl hæmometer (see page 176).

4. **Tallquist's Hæmoglobin Scale.**—This consists essentially of a series of ten colors corresponding to stains produced by blood containing varying percentages of hæmoglobin. In using this scale a drop of blood is allowed to fall on a small section of filter paper and the resulting color is compared with the ten colors of the scale. When the color in the scale is found which corresponds to the color of the blood stain the accompanying hæmoglobin value is read off directly. This is a very convenient method for determining hæmoglobin at the bedside. There is a possibility of the colors being inaccurately printed, however, and even if originally correct in tint, under the continued influence of air and light they must eventually alter somewhat.

5. **Thoma-Zeiss Hæmocytometer.**—This is an instrument used in "blood counting," *i. e.*, in determining the number of erythrocytes and leucocytes. The instrument consists

of a microscopic slide constructed of heavy glass and provided with a central counting cell (see Fig. 70, below). This cell, with the cover-glass in position, is exactly 0.1 millimeter deep. The floor of the cell is divided by delicate lines into squares each of which is $\frac{1}{400}$ of a square millimeter in area (see Fig. 72, p. 183). The volume of blood therefore between any particular square and the cover glass above must be $\frac{1}{4000}$ cubic

FIG. 70.

THOMA-ZEISS COUNTING CHAMBER. (*Da Costa*.)

millimeter. Accompanying each instrument are two capillary pipettes (Fig. 71, p. 182), each constructed with a mixing bulb in its upper portion. Each bulb is further provided with an enclosed glass bead which is of great assistance in mixing the contents of the chamber. The stem of each pipette is graduated in tenths from the tip to the bulb. The final graduation at the upper end of the bulb is 101 on the pipette used in mixing the blood sample in which the erythrocytes are counted (erythrocytometer, see Fig. 71, p. 182), and 11 on the pipette used in mixing the blood sample for the leucocyte count (leucocytometer, see Fig. 71, p. 182). In making "blood counts" with the hæmocytometer it is necessary to use some diluting fluid. Two very satisfactory forms of fluid for this purpose are Toison's and Sherrington's solutions.¹ When

¹ Toison's solution has the following formula:

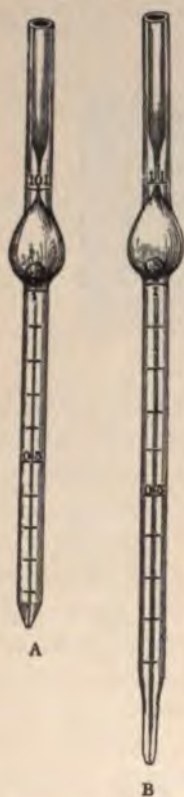
Methyl violet	0.025 gram.
Sodium chloride	1 gram.
Sodium sulphate	8 grams.
Glycerin	30 grams.
Distilled water	160 grams.

Sherrington's solution has the following formula:

Methylene-blue	0.1 gram.
Sodium chloride	1.2 gram.
Neutral potassium oxalate	1.2 gram.
Distilled water	300.0 grams.

either of these solutions is used as the diluting fluid it is possible to make a very satisfactory count of both the erythrocytes and leucocytes from the same preparation, since the leucocytes are stained by the methyl violet or methylene-blue.

FIG. 71.



THOMA-ZEISS CAP-
ILLARY PIPETTES.
A, Erythrocytom-
eter; B, leuco-
cytometer.

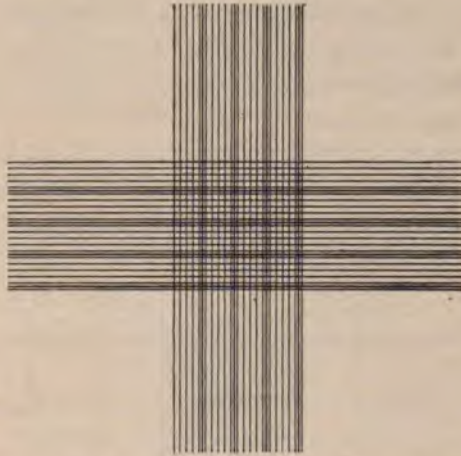
In counting the erythrocytes by means of the hæmocytometer proceed as follows: Thoroughly cleanse the tip of the finger or lobe of the ear of the subject by the use of soap and water, alcohol and ether applied in the sequence just given. Puncture the skin by means of a needle or scalpel and allow the blood drop to form without pressure. Place the tip of the pipette in contact with the blood drop, being careful to avoid touching the skin, and draw blood into the pipette up to the point marked 0.5 or 1 according to the desired dilution. Rapidly wipe the tip of the pipette and immediately fill it to the point marked 1.01 with Toison's or Sherrington's solution. Now thoroughly mix the blood and diluting fluid within the mixing chamber by tapping the pipette gently against the finger or by shaking it while held securely with the thumb at one end and the middle finger at the other. After the two fluids have been thoroughly mixed the diluting fluid contained in the capillary-tube below the bulb should be discarded in order to insure the collection of a drop of the thoroughly mixed blood and diluting solution for examination. Transfer a drop from the pipette to the ruled floor of the counting chamber and,

after placing the cover-glass firmly in position,¹ allow an

¹ If the cover glass is in accurate apposition to the counting cell Newton's rings may be plainly observed.

interval of a few minutes to elapse for the corpuscles to settle before making the count. Now place the slide under the microscope and count the number of erythrocytes in a number of squares, counting the corpuscles which are in contact with the upper and the right hand boundaries of the square as belonging to that square. Take the squares in some definite

FIG. 72.

ORDINARY RULING OF THOMA-ZEISS COUNTING CHAMBER. (*Da Costa.*)

sequence in order that the recounting of the same corpuscles may be avoided. Of course, all things being equal, the greater the number of squares examined the more accurate the count. It is considered essential under all circumstances, where an accurate count is desired, that the counting chamber shall be filled at least twice and the individual counts made in each instance, as indicated above, before the data are deemed satisfactory.

To calculate the number of erythrocytes per cubic millimeter of undiluted blood proceed as follows: Determine the number of corpuscles in any given number of squares and divide this total by the number of squares, thus obtaining the average number of erythrocytes per square. Multiply this average by 4,000 to obtain the number of erythrocytes per

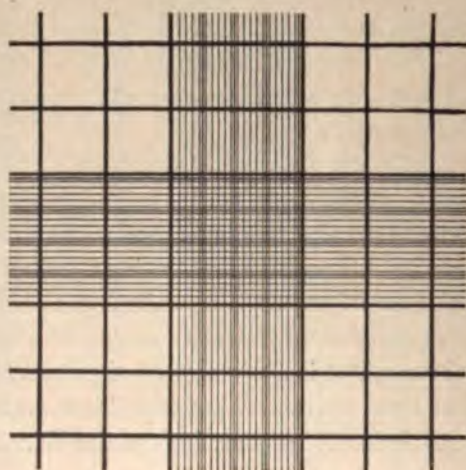
cubic millimeter of *diluted* blood, and multiply this product by 100 or 200, according to the dilution, to obtain the number of erythrocytes per cubic millimeter of *undiluted* blood. Thus:

$$\text{Average number of erythrocytes per square} \times 4,000 \times 200 \text{ (or 100)} = \text{Number of erythrocytes per cubic millimeter.}$$

Great care should be taken to see that the capillary pipette is properly cleaned. After using, it should be immediately rinsed out with the diluting fluid, then with water, alcohol and ether in the sequence given. Finally dry air should be drawn through the capillary and a horse hair inserted to prevent the entrance of dust particles.

In counting leucocytes by means of the hæmocytometer proceed as follows: As mentioned above, if the diluting fluid is either Toison's or Sherrington's solution the leucocytes may

FIG. 73.



ZAPPERT'S MODIFIED RULING OF THOMA-ZEISS COUNTING CHAMBER. (*Da Costa.*)

be counted in the same specimen of blood in which the erythrocytes are counted. When this is done it is customary to use a slide provided with Zappert's modified ruling (Fig. 73, above). This method is rather more accurate than the older

one of counting the leucocytes in a separate specimen of blood. Furthermore it is obviously preferable to count both the erythrocytes and the leucocytes from the same blood sample. To insure accuracy the number of leucocytes within the whole ruled region should be determined in *duplicate* blood samples. This includes the examination of an area eighteen times as great as the old style Thoma-Zeiss central ruling. This region then would correspond to 3,600 of the small squares and if *duplicate* examinations were made the total number of small squares examined would aggregate 7,200. The calculation would be as follows:

$$\frac{\text{Number of leucocytes in}}{7,200 \text{ squares}} \times 200 \times 4,000 \div 7,200 = \frac{\text{Number of leucocytes per}}{\text{cubic millimeter.}}$$

If a Zappert slide is not available a good plan to follow is to place a diaphragm in the tube of the ocular of the microscope consisting of a circle of black cardboard or metal¹ having a square hole in its centre of such a size as to allow of the examination of exactly 100 squares or one-fourth of a square millimeter at one time. With this arrangement any portion of the specimen may be examined and counted whether within or without the ruled area. In counting by means of this device it is of course helpful if the microscope is provided with a mechanical stage, but even without this arrangement, if the observer is careful to see that the leucocytes at the extreme boundary of one field move to the opposite boundary when the position of the slide is changed, the device may be very satisfactorily employed. The leucocytes should be counted in 36 of the diaphragm-fields in *duplicate* specimens and the calculation made in the same manner as explained above.

If the leucocytes are counted in a separate specimen of blood ordinarily the diluting fluid is 0.3–0.5 per cent acetic acid, a fluid in which the leucocytes alone remain visible. Under these conditions the dilution is customarily made in the pipette having 11 as the final graduation. The capillary

¹ Ehrlich's mechanical eye-piece is also very satisfactory for this purpose.

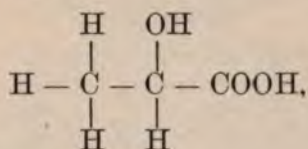
portion is of larger caliber and so requires a greater amount of blood to fill it to the 0.5 or 1 mark than is required in the use of the other form of pipette. In counting the leucocytes according to this method it is customary to draw blood into the pipette up to the 1 mark and immediately fill the remaining portion of the apparatus to the 11 graduation with the 0.3-0.5 per cent acetic acid. It then remains to count the number of leucocytes in the whole central ruled portion of 400 squares. This should be done in *duplicate* samples and the calculation made as follows:

$$\frac{\text{Number of leucocytes in}}{800 \text{ squares}} \times 4,000 \times 10 \div 800 = \frac{\text{Number of leucocytes per}}{\text{cubic millimeter.}}$$

CHAPTER XII.

MILK.

Milk is the most satisfactory individual food material elaborated by nature. It contains the three nutrients, proteid, fat and carbohydrate and inorganic salts in such proportion as to render it a very acceptable dietary constituent. It is a specific product of the secretory activity of the mammary gland. It contains, as the principal solids, *tri-olein*, *tri-palmitin*, *tri-stearin*, *tri-butylin*, *caseinogen*, *lactalbumin*, *lacto-globulin*, *lactose* and *calcium phosphate*. It also contains at least traces of *lecithin*, *cholesterin*, *urea*, *creatin*, *creatinin* and the tri-glycerides of caproic, lauric and myristic acids. Fresh milk is amphoteric in reaction, but upon standing for a sufficiently long time, unsterilized, it becomes acid in reaction, due to the production of fermentation lactic acid,

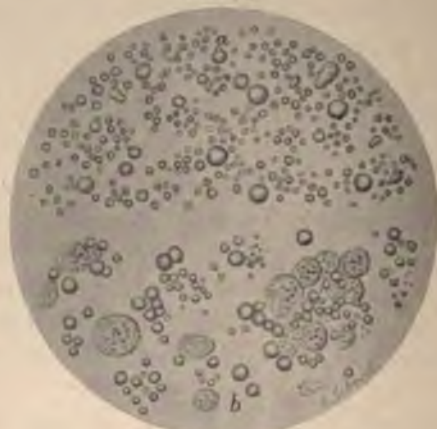


from the lactose contained in it. This is brought about through bacterial activity. The white color is imparted to the milk partly through the fine emulsion of the fat and partly through the medium of the caseinogen in solution. The specific gravity of milk varies somewhat, the average being about 1.030. Its freezing-point is about -0.56°C .

Fresh milk does not coagulate on being boiled but a film consisting of a combination of caseinogen forms on the surface. If the film be removed, thus allowing a fresh surface to come in contact with the air, a new film will form indefinitely upon the application of heat. Surface evaporation and the presence

of fat facilitate the formation of the film but are not essential. (Rettger.) If the milk is acid in reaction, through the inception of lactic acid fermentation, or from any other cause, no film will form when heat is applied, but instead a true coagulation will occur. The milk-curdling enzymes of the gastric and the pancreatic juice have the power of splitting the caseinogen of the milk, through a process of hydrolysis, into *soluble casein*

FIG. 74.



NORMAL MILK AND COLOSTRUM.
a, Normal milk; b, Colostrum.

and a *peptone-like* body. This soluble casein then forms a combination with the calcium of the milk and an insoluble curd of *calcium casein* or *casein* results. The clear fluid surrounding the curd is known as *whey*.

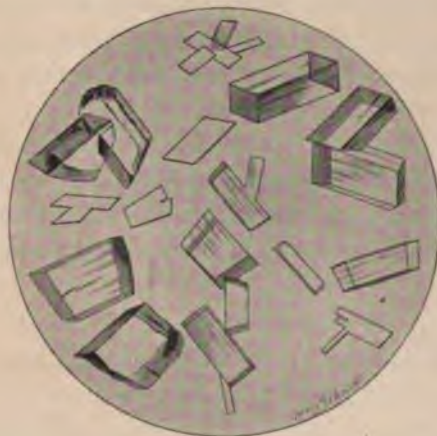
The most pronounced difference between human milk and cow's milk is in the proteid content, although there are also differences in the fats and likewise striking biological differences difficult to define chemically. It has been shown that the caseinogen of human milk differs from the caseinogen of cow's milk in being more difficult to precipitate by acid or coagulate by rennin. The casein curd also forms in a much looser and more flocculent manner than that from cow's milk and is for this reason much more easily digested than the

latter. Interesting data relative to the composition of milk from various sources, may be gathered from the following table which was compiled mainly from the results of investigations by Bunge and by Abderhalden. It will be noted that the composition of the milk varies directly with the length of time needed for the young of the particular species to double in weight.

Species.	Period in which weight of the new-born is doubled (days).	100 parts of milk contain			
		Proteids.	Salts.	Calcium.	Phosphoric acid
Man, ...	180	1.6	0.2	0.033	0.047
Horse.....	60	2.0	0.4	0.124	0.131
Cow.....	47	3.5	0.7	0.160	0.197
Goat.....	22	3.7	0.8	0.197	0.284
Sheep.....	15	4.9	0.8	0.245	0.293
Pig.....	14	5.2	0.8	0.249	0.308
Cat.....	9.5	7.0	1.0	—	—
Dog.....	9	7.4	1.3	0.455	0.508
Rabbit.....	6	10.4	2.5	0.891	0.997

Lactose, the carbohydrate constituent of milk, is an important member of the disaccharide group. It occurs only in

FIG. 75.



LACTOSE.

milk, except as it is found in the urine of women during pregnancy, during the nursing period and soon after weaning; it also

occurs in the urine of normal persons after the ingestion of a very large amount of lactose in the food. It is not derived directly from the blood but is a specific product of the cellular activity of the mammary gland. It has strong reducing power, is dextro-rotatory and forms an osazone with phenylhydrazin. The souring of milk is due to the formation of lactic acid from lactose through the agency of the *bacterium lactis*. Putrefactive bacteria in the alimentary canal may bring about this same reaction. Lactose is *not* fermentable by pure yeast.

Caseinogen, the principal proteid constituent of milk belongs to the group of phospho-proteids. It has acidic properties and combines with bases to produce salts. It is not coagulable upon boiling and is precipitated from its neutral solution by certain metallic salts as well as upon saturation with sodium chloride or magnesium sulphate. Its acid solution is precipitated by an excess of mineral acid.

Lactalbumin and lactoglobulin, the other proteid constituents of milk, closely resemble serum albumin and serum globulin in their general properties.

Colostrum is the name given to the product of the mammary gland secreted for a short time before parturition and during the early period of lactation (see Fig. 74, p. 188). It is yellowish in color, contains more solid matter than ordinary milk and has a higher specific gravity (1.040-1.080). The most striking difference between colostrum and ordinary milk is the high percentage of lactalbumin and lactoglobulin in the former. This abnormality in the proteid content is responsible for the coagulation of colostrum upon boiling.

EXPERIMENTS ON MILK.

1. **Reaction.**—Test the reaction of fresh cow's milk to litmus.
2. **Biuret Test.**—Make the biuret test according to directions given on page 45.
3. **Microscopical Examination.**—Examine fresh *whole* milk, *skimmed* or *centrifugated* milk and *colostrum* under the

microscope. Compare the microscopical appearance with Fig. 74, page 188.

4. **Specific Gravity.**—Determine the specific gravity of both whole and skimmed milk. Which possesses the higher specific gravity? Explain why this is so.

5. **Film Formation.**—Place 10 c.c. of milk in a small beaker and boil a few minutes. Note the formation of a film. Remove the film and heat again. Does the film now form? Of what substance is this film composed? The biuret test was positive, why do we not get a coagulation here when we heat to boiling?

6. **Coagulation Test.**—Place about 5 c.c. of milk in a test-tube, acidify slightly with dilute acetic acid and heat to boiling. Do you get any coagulation? Why?

7. **Action of Hot KOH.**—To a little milk in a test-tube add a few drops of KOH and heat. A yellow color develops and gradually deepens into a brown. To what is the formation of this color due?

8. **Test for Chlorides.**—To about 5 c.c. of milk in a test-tube add a few drops of *very dilute* nitric acid to form a precipitate. Filter off this precipitate and test the filtrate for chlorides. Does milk contain any chlorides?

9. **Guaiaic Test.**—To about 5 c.c. of water in a test-tube add 3 drops of milk and enough alcoholic solution of guaiac (strength about 1:60)¹ to cause a turbidity. Thoroughly mix the fluids by shaking and observe any change which may gradually take place in the color of the mixture. If no blue color appears in a short time, heat the tube gently below 60° C. and observe whether the color reaction is hastened. In case a blue color does not appear in the course of a few minutes, add hydrogen peroxide or old turpentine, drop by drop, until the color is observed. Fresh milk will frequently give this blue color when treated with an alcoholic solution of

¹ Buckmaster advises the use of an alcoholic solution of guaiaconic acid instead of an alcoholic solution of guaiac resin. Guaiaconic acid is a constituent of guaiac resin.

gum guaiac without the addition of hydrogen peroxide or old turpentine. See discussion on page 158.

10. **Saturation with MgSO_4 .**—Place about 5 c.c. of milk in a test-tube and saturate with solid magnesium sulphate. What is this precipitate?

11. **Influence of Rennin on Milk.**—Prepare a series of five tubes as follows:

(a) 5 c.c. of fresh milk + 0.2 per cent HCl (add drop by drop until a precipitate forms).

(b) 5 c.c. of fresh milk + 5 drops of *rennin* solution.

(c) 5 c.c. of fresh milk + 10 drops of 0.5 per cent Na_2CO_3 .

(d) 5 c.c. of fresh milk + 10 drops of ammonium oxalate.

(e) 5 c.c. of fresh milk + 5 drops of 0.2 per cent HCl.

Now to each of the tubes (c), (d) and (e) add 5 drops of *rennin* solution. Place the whole series of five tubes at 40°C . and after 10–15 minutes note what is occurring in the different tubes. Give a reason for each particular result.

12. **Preparation of Caseinogen.**—Fill a large beaker one-third full of *skimmed* (or centrifugated) milk and dilute it with an equal volume of water. Add dilute hydrochloric acid until a flocculent precipitate forms. Stir after each acidification and do not add an excess of the acid as the precipitate would dissolve. Allow the precipitate to settle, decant the supernatant fluid and reserve it for use in later (13–15) experiments. Filter off the precipitate of caseinogen and remove the excess of moisture by pressing it between filter papers. Transfer the caseinogen to a small beaker, add enough 95 per cent alcohol to cover it and stir for a few moments. Filter, and press the precipitate between filter papers to remove the alcohol. Transfer the caseinogen again to a small *dry* beaker, cover the precipitate with ether and heat on a water-bath for ten minutes, stirring continuously. Filter (reserve the filtrate), and press the precipitate as dry as possible between filter papers. Open the papers and allow the ether to evaporate spontaneously. Grind the precipitate to a powder in a mortar. Upon the caseinogen prepared in this way make the following tests:

(a) *Solubility*.—Try the solubility in the ordinary solvents.

(b) *Millon's Reaction*.—Make the test according to the directions given on page 44.

(c) *Biuret Test*.—Make the test according to the directions given on page 45.

(d) *Xanthoproteic Reaction*.—Make the test according to the directions given on page 44.

(e) *Loosely Combined Sulphur*.—Test for loosely combined sulphur according to the directions given on page 52.

(f) *Fusion Test for Phosphorus*.—Test for phosphorus by fusion according to directions given on page 223.

13. **Coagulable Proteids of Milk**.—Place the filtrate from the original caseinogen precipitate in a casserole and heat, on a wire gauze, over a free flame. As the solution concentrates, a coagulum consisting of *lactalbumin* and *lactoglobulin* will form. Continue to concentrate the solution until the volume is about one-half that of the original solution. Filter off the coagulable proteids (reserve the filtrate) and test them as follows:

(a) *Millon's Reaction*.—Make the test according to the directions given on page 44.

(b) *Biuret Test*.—Make the test according to the directions given on page 45.

(c) *Xanthoproteic Reaction*.—Make the test according to the directions given on page 44.

14. **Detection of Calcium Phosphate**.—Evaporate the filtrate from the coagulable proteids, on a water-bath, until crystals begin to form. It may be necessary to concentrate to 15 c.c. before any crystallization will be observed. Cool the solution, filter off the crystals (reserve the filtrate) and test them as follows:



CALCIUM PHOSPHATE.

(a) *Microscopical Examination*.—Examine the crystals and compare them with those in Fig. 76, above.

(b) Dissolve the crystals in nitric acid. Test part of the acid solution for phosphates. Render the remainder of the solution slightly alkaline with ammonia, then acidify with acetic acid and add ammonium oxalate. Examine the crystals under the microscope and compare them with those in Fig. 99, p. 320.

15. Detection of Lactose.—Concentrate the filtrate from the calcium phosphate until it is of a syrup-like consistency. Allow it to stand over night and observe the formation of crystals of lactose. Make the following experiments:

(a) *Microscopical Examination.*—Examine the crystals and compare them with those in Fig. 75, page 189.

(b) *Fehling's Test.*—Try Fehling's test upon the mother liquor.

(c) *Phenylhydrazin Test.*—Apply the phenylhydrazin test to some of the mother liquor according to the directions given on page 5.

16. Milk Fat.—(a) Evaporate the ether filtrate from the caseinogen (Experiment 12) and observe the fatty residue. The milk fat was carried down with the precipitate of caseinogen and was removed when the latter was treated with ether. If centrifugated milk was used in the preparation of the caseinogen the amount of fat in the ether filtrate may be very small. To secure a larger yield of fat proceed according to directions given under (b) below.

(b) To 25 c.c. of whole milk in an evaporating dish add a little sand or filter paper and evaporate the fluid to dryness on a water-bath. Grind or break up the residue after cooling and extract with ether in a flask. Filter and remove the ether from the filtrate by evaporation. How can you identify fats in the ethereal residue?

17. Saponification of Butter.—Dissolve a small amount of butter in alcohol made strongly alkaline with potassium hydroxide. Place the alcoholic-potash solution in a casserole, add about 100 c.c. of water and boil for 10–15 minutes or until the odor of alcohol cannot be detected. Place the casserole in a hood and neutralize the solution with sulphuric

acid. Note the odor of volatile fatty acids particularly *butyric acid*.

18. **Detection of Preservatives.**—(a) *Formaldehyde*.

I. *Gallic Acid Test*.—Acidify 30 c.c. of milk with 2 c.c. of normal sulphuric acid and distil. Add 0.2–0.3 c.c. of a saturated alcoholic solution of gallic acid to the *first* 5 c.c. of the distillate, then incline the test-tube and slowly introduce 3–5 c.c. of concentrated sulphuric acid, allowing it to run slowly down the side of the tube. A green ring, which finally changes to blue, is formed at the juncture of the fluids. This is claimed, by Sherman, to be twice as delicate as either the sulphuric acid or the hydrochloric acid test for formaldehyde.

II. *Hydrochloric Acid Test*.—Mix 10 c.c. of milk and 10 c.c. of concentrated hydrochloric acid containing about 0.002 gram of ferric chloride in a small porcelain evaporating dish or casserole and *gradually* raise the temperature of the mixture nearly to the boiling-point, with occasional stirring. If formaldehyde is present a *violet* color is produced, while a brown color develops in the absence of formaldehyde. In case of doubt the mixture, after having been heated nearly to the boiling-point for about one minute, should be diluted with 50–75 c.c. of water, and the color of the diluted fluid carefully noted, since the violet color if present will quickly disappear. Formaldehyde may be detected by this test when present in the proportion 1:250,000.

(b) *Salicylic Acid and Salicylates*.—Remont's Method.¹ Acidify 20 c.c. of milk with sulphuric acid, shake well to break up the curd, add 25 c.c. of ether, mix thoroughly and allow the mixture to stand. By means of a pipette remove 5 c.c. of the ethereal extract, evaporate it to dryness, boil the residue with 10 c.c. of 40 per cent alcohol and cool the alcoholic solution. Make the volume 10 c.c., filter through a *dry* paper if necessary to remove fat, and to 5 c.c. of the filtrate, which represents 2 c.c. of milk, add 2 c.c. of a 2 per cent solution of ferric chloride. The production of a *purple* or *violet* color indicates the presence of salicylic acid.

¹ Sherman's Organic Analysis, p. 232.

This test may form the basis of a quantitative method by diluting the final solution to 50 c.c. and comparing this with standard solutions of salicylic acid. The colorimetric comparisons may be made in a Duboscq colorimeter.

(c) *Hydrogen Peroxide*.—Add 2-3 drops of a 2 per cent aqueous solution of paraphenylenediamine hydrochloride to 10-15 c.c. of milk. If hydrogen peroxide is present a *blue* color will be produced immediately upon shaking the mixture or after allowing it to stand for a few minutes. It is claimed that hydrogen peroxide may be detected by this test when present in the proportion 1:40,000.

(d) *Boric Acid and Borates*.—To the ash, obtained according to the directions given on p. —, add 2 drops of dilute hydrochloric acid and 1 c.c. of water. Place a strip of turmeric paper in the dish and after allowing it to soak for about one minute remove it and allow it to dry in the air. The presence of boric acid is indicated by the production of a deep *red* color which changes to *green* or *blue* upon treatment with a dilute alkali. This test is supposed to show boric acid when present in the proportion 1:8000.

CHAPTER XIII.

EPITHELIAL AND CONNECTIVE TISSUES.

EPITHELIAL TISSUE (KERATIN).

The albuminoid *keratin* constitutes the major portion of hair, horn, hoof, feathers, nails and the epidermal layer of the skin. There is a group of keratins the members of which possess very similar properties. The keratins as a group are insoluble in the usual proteid solvents and are not acted upon by the gastric or pancreatic juices. They all respond to the xanthoproteic and Millon reactions and are characterized by containing large amounts of sulphur. Keratin from any of its sources may be prepared in a pure form by treatment, in sequence, with artificial gastric juice, artificial pancreatic juice, boiling alcohol and boiling ether, from twenty-four to forty-eight hours being devoted to each process.

EXPERIMENTS ON EPITHELIAL TISSUE.

Keratin.

Horn shavings may be used in the experiments which follow:

1. *Solubility*.—Test the solubility of keratin in the ordinary solvents (see p. 4).
2. *Millon's Reaction*.
3. *Xanthoproteic Reaction*.
4. *Adamkiewicz's Reaction*.
5. *Hopkins-Cole Reaction*.
6. *Test for Loosely Combined Sulphur*.

CONNECTIVE TISSUE.

I. WHITE FIBROUS TISSUE.

The principal solid constituent of white fibrous connective tissue is the albuminoid collagen. This body is also found in

smaller percentage in cartilage, bone and ligament, but the collagen from the various sources is not identical in composition. In common with the keratins, collagen is insoluble in the usual proteid solvents. It differs from keratin in containing less sulphur. One of the chief characteristics of collagen is the property of being hydrolyzed by boiling acid or water with the formation of *gelatin*. It gives Millon's reaction as well as the xanthoproteic and biuret tests.

The form of white fibrous tissue most satisfactory for general experiments is the *tendo Achillis* of the ox. According to Buerger and Gies the fresh tissue has the following composition:

Water	62.87%
Solids	37.13
Inorganic matter	0.47
Organic matter	36.66
Fatty substance (ether-soluble).....	1.04
Coagulable proteid	0.22
Mucoid	1.28
Elastin	1.63
Collagen	31.59
Extractives, etc.	0.90

The mucoid mentioned above is called *tendomucoid* and is a glucoproteid. It possesses properties similar to those of other connective tissue mucoids, *e. g.*, osseomucoid and chondromucoid.

Gelatin, the body which results from the hydrolysis of collagen, is also an albuminoid. It responds to nearly all the proteid tests. It differs from the keratins and collagen in being easily digested and absorbed. Gelatin is not a satisfactory substitute for the proteid constituents of a normal diet however, since a certain portion of its nitrogen is not available for the uses of the organism. Gelatin from cartilage differs from the gelatin from other sources in containing a lower percentage of nitrogen.

EXPERIMENTS ON WHITE FIBROUS TISSUE.

The *tendo Achillis* of the ox may be taken as a satisfactory type of the white fibrous connective tissue.

1. **Preparation of Tendomucoid.**—Dissect away the fascia from about the tendon and cut the clean tendon into small pieces. Wash the pieces in water, changing the wash water often in order to remove as much as possible of the soluble proteid and inorganic salts. Transfer the washed pieces of tendon to a flask and add 300 c.c. of *half-saturated* lime-water.¹ Shake the flask at intervals for twenty-four hours. Filter off the pieces of tendon and precipitate the mucoid with dilute hydrochloric acid. Allow the mucoid precipitate to settle, decant the supernatant fluid and filter the remainder. Test the mucoid as follows:

(a) *Solubility.*—Try the solubility in the ordinary solvents (see p. 4).

(b) *Biuret Test.*—First dissolve the mucoid in KOH solution and then add a dilute solution of CuSO_4 .

(c) *Test for Loosely Combined Sulphur.*

(d) *Hydrolysis of Tendomucoid.*—Place the remainder of the mucoid in a small beaker, add about 30 c.c. of water and 2 c.c. of dilute hydrochloric acid and boil until the solution becomes dark brown. Cool the solution, neutralize it with *solid* KOH and test by Fehling's test. With a reduction of Fehling's solution and a positive biuret test what do you conclude regarding the nature of tendomucoid?

2. **Collagen.**—This substance is present in the tendon to the extent of about 32 per cent. Therefore in making the following tests upon the pieces of tendon from which the mucoid, soluble proteid and inorganic salts were removed in the last experiment, we may consider the tests as being made upon *collagen*.

(a) *Solubility.*—Cut the collagen into very fine pieces and try its solubility in the ordinary solvents (see page 4).

(b) *Millon's Reaction.*

(c) *Biuret Test.*

(d) *Xanthoproteic Reaction.*

¹ Made by mixing equal volumes of *saturated* lime-water and water from the faucet.

(e) *Hopkins-Cole Reaction.*

(f) *Test for Loosely Combined Sulphur.*—Take a large piece of collagen in a test-tube and add about 5 c.c. of KOH solution. Heat until the collagen is partly decomposed, then add 1–2 drops of plumbic acetate and again heat to boiling.

(g) *Hydrolysis of Collagen.*—Transfer the remainder of the pieces of collagen to a casserole, fill the vessel about two-thirds full of water and boil for several hours, adding water at intervals as needed. By this means the collagen is *hydrolyzed* and a body known as *gelatin* is formed.

3. **Gelatin.**—On the gelatin formed from the hydrolysis of collagen in the above experiment (g), or on gelatin furnished by the instructor make the following tests:

(a) *Solubility.*—Try the solubility in the ordinary solvents (see page 4) and in *hot* water.

(b) *Millon's Reaction.*

(c) *Hopkins-Cole Reaction.*—Conduct this test according to the modification given on page 51.

(d) *Test for Loosely Combined Sulphur.*

Make the following tests upon a *solution* of gelatin in hot water:

(a) *Precipitation by Mineral Acids.*—Is it precipitated by strong mineral acids such as concentrated hydrochloric acid?

(b) *Salting-Out Experiment.*—Saturate a little of the solution with solid ammonium sulphate. Is the gelatin precipitated? Repeat the experiment with sodium chloride. What is the result?

(c) *Precipitation by Metallic Salts.*—Is it precipitated by metallic salts such as cupric sulphate, mercuric chloride and plumbic acetate?

(d) *Coagulation Test.*—Does it coagulate upon boiling?

(e) *Precipitation by Alkaloidal Reagents.*—Is it precipitated by such reagents as picric acid, tannic acid and trichloroacetic acid?

(f) *Biuret Test.*—Does it respond to the biuret test?

(g) *Precipitation by Alcohol*.—Fill a test-tube one-half full of 95 per cent alcohol and pour in a small amount of *concentrated* gelatin solution. Do you get a precipitate? How would you prepare pure gelatin from the *tendo Achillis* of the ox?

II. YELLOW ELASTIC TISSUE (ELASTIN).

The *Ligamentum nuchæ* of the ox may be taken as a satisfactory type of the yellow elastic connective tissue. The principal solid constituent of this tissue is *elastin*, a member of the albuminoid group. In common with the keratins and collagen, elastin is an insoluble body and gives the proteid color reactions. It differs from keratin principally in the fact that it may be digested by enzymes and that it contains a very small amount of sulphur.

Yellow elastic tissue also contains mucoid and collagen but these are present in much smaller amount than in white fibrous tissue, as may be seen from the following percentage composition of the fresh *ligamentum nuchæ* of the ox as determined by Vandegrift and Gies:

Water	57.57%
Solids	42.43
Inorganic matter	0.47
Organic matter	41.96
Fatty substance (ether-soluble)	1.12
Coagulable proteid	0.62
Mucoid	0.53
Elastin	31.67
Collagen	7.23
Extractives, etc.	0.80

EXPERIMENTS ON ELASTIN.

1. *Preparation of Elastin (Richards and Gies)*.—Cut the ligament into fine strips, run it through a meat chopper and wash the finely divided material in cold, running water for 24-48 hours. Add an excess of *half-saturated* lime-water (see note at bottom of p. 199) and allow the hashed ligament

to extract for 48-72 hours. Decant the lime-water, remove all traces of alkali by washing in water and then boil in water with repeated renewals until only traces of proteid material can be detected in the wash water. Decant the fluid and boil the ligament in 10 per cent acetic acid for a few hours. Treat the pieces with 5 per cent hydrochloric acid at room temperature for a similar period, extract again in *hot* acetic acid and in *cold* hydrochloric acid. Wash out traces of acid by means of water and then thoroughly dehydrolyze by boiling alcohol and boiling ether in turn. Dry in an air-bath and grind to a powder in a mortar.

2. **Solubility.**—Try the solubility of the finely divided elastin, prepared by yourself or furnished by the instructor, in the ordinary solvents (see page 4). How does its solubility compare with that of collagen?

3. **Millon's Reaction.**

4. **Xanthoproteic Reaction.**

5. **Biuret Test.**

6. **Hopkins-Cole Reaction.**—Conduct this test according to the modification given on page 51.

7. **Test for Loosely Combined Sulphur.**

III. CARTILAGE.

The principal solid constituents of the matrix of cartilaginous tissue are *chondromucoid*, *chondroitin-sulphuric acid*, *chondroalbumoid* and *collagen*. Chondromucoid differs from the mucoids isolated from other connective tissues in the large amount of chondroitin-sulphuric acid obtained upon decomposition. Besides being an important constituent of all forms of cartilage, chondroitin-sulphuric acid has been found in bone, ligament, the mucosa of the pig's stomach, the kidney of the ox, the inner coats of large arteries and in human urine. It may be decomposed through the action of acid and yields a nitrogenous body known as *chondroitin* and later this body yields *chondrosin*. Chondrosin is also a nitrogenous body and has the power of reducing Fehling's solution more strongly

than dextrose. Sulphuric acid is a by-product in the formation of chondroitin, and acetic acid is a by-product in the formation of chondrosin.

Chondroalbumoid is similar in some respects to elastin and keratin. It differs from keratin in being soluble in gastric juice and in containing considerably less sulphur than any member of the keratin group. It gives the usual proteid color reactions.

EXPERIMENTS ON CARTILAGE.

1. **Preparation of the Cartilage.**—Boil the trachea of an ox in water until the cartilage rings may be completely freed from the surrounding tissue. Use the cartilage so obtained in the following experiments.

2. **Solubility.**—Cut one of the rings into very small pieces and try the solubility of the cartilage in the ordinary solvents (see page 4).

3. **Millon's Reaction.**

4. **Xanthoproteic Reaction.**

5. **Hopkins-Cole Reaction.**—Conduct this test according to the modification given on page 51.

6. **Test for Loosely Combined Sulphur.**

7. **Preparation of Cartilage Gelatin.**—Cut the remaining cartilage rings into small pieces, place them in a casserole with water and boil for several hours. Filter while the solution is still hot. Observe that the filtrate soon becomes more or less solid. What is the reason for this? Bring a portion of the material into solution by heat and try the following tests:

(a) *Biuret Test.*

(b) *Test for Loosely Combined Sulphur.*

(c) To about 5 c.c. of the solution in a test-tube add a few drops of barium chloride. Do you get a precipitate, and if so to what is the precipitate due?

(d) To about 5 c.c. of the solution in a test-tube add a few drops of dilute hydrochloric acid and boil for a few moments. Now add a little barium chloride to this solution. Is the precipitate any larger than that obtained in the preceding experiment? Why?

(e) To the remainder of the solution add a little dilute hydrochloric acid and boil for a few moments. Cool the solution, neutralize with *solid* KOH and try Fehling's test. Explain the result.

IV. OSSEOUS TISSUE.

Bone is composed of about equal parts of organic and inorganic matter. The organic portion, called *ossein*, may be obtained by removing the inorganic salts through the medium of dilute acid. Ossein is practically the same body which is termed collagen in the other connective tissues, and in common with collagen may be hydrolyzed with weak acids to form gelatin.

In common with the other connective tissues bone contains a mucoid and an albumoid. Because of their origin these bodies are called *osseomucoid* and *osseoalbumoid*. Osseomucoid, when boiled with hydrochloric acid, yields sulphuric acid and a substance capable of reducing Fehling's solution. The composition of osseomucoid is very similar to that of tendomucoid and chondromucoid (see page 62).

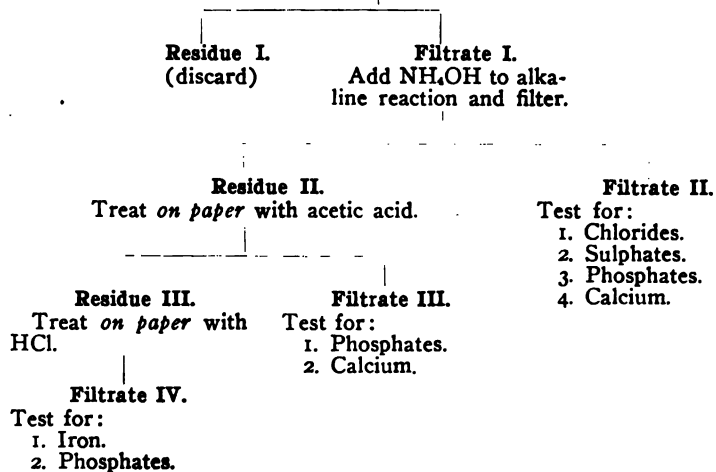
EXPERIMENT ON OSSEOUS TISSUE.

Analysis of Bone Ash.—Take one gram of bone ash in a small beaker and add a little dilute nitric acid. What does the effervescence indicate? Stir thoroughly and when the major portion of the ash is dissolved add an equal volume of water and filter. To the acid filtrate add ammonium hydroxide to alkaline reaction. A heavy white precipitate of phosphates results. (What phosphates are precipitated here by the ammonia?) Filter and test the filtrate for chlorides, sulphates, phosphates and calcium. Add dilute acetic acid to the precipitate on the paper and test this filtrate for calcium and phosphates. To the precipitate remaining undissolved on the paper add a little dilute hydrochloric acid and test this last filtrate for phosphates and iron.

Reference to the following scheme may facilitate the analysis.

BONE ASH.

Add dilute HNO_3 , stir thoroughly and after the major portion of the ash has been brought into solution add a little distilled water and filter.

**V. ADIPOSE TISSUE.**

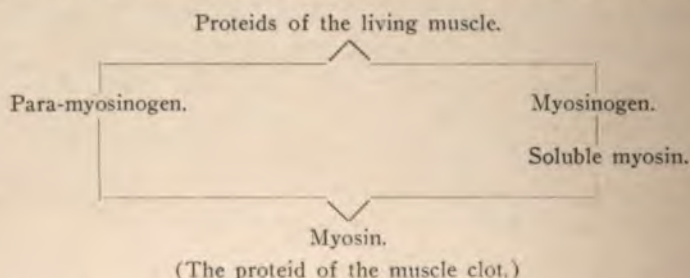
For discussion and experiments see the chapter on Fats, page 96.

CHAPTER XIV.

MUSCULAR TISSUE.

The muscular tissues are divided physiologically into the voluntary and the involuntary. In the chemical examination of muscular tissue the voluntary form is generally employed. Muscle contains about 25 per cent of solid matter, of which about four-fifths is proteid material and the remaining one-fifth extractives and inorganic salts.

The proteids are the most important of the constituents of muscular tissue. In the living muscle we find two proteids, *myosinogen* and *para-myosinogen*. These may be shown to be present in *muscle plasma* expressed from fresh muscles. In common with the plasma of the blood this muscle plasma has the power of coagulating, and the clot formed in this process is called *myosin*. In the onset of *rigor mortis* we have an indication of the formation of this myosin clot within the body. The relation between the proteids of *living* and *dead* muscle is represented graphically by Halliburton as follows :



Of the total proteid content of living muscle about 75 per cent is made up by the *myosinogen* and the remaining 25 per cent is *para-myosinogen*. These proteids may be separated by subjecting the muscle plasma to fractional coagulation in the usual way. Under these conditions the *para-myosinogen*

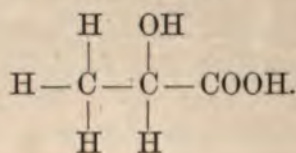
is found to coagulate at 47° C. and the myosinogen to coagulate at 56° C. It is also claimed by some investigators that it is possible to separate these two proteids by the fractional ammonium sulphate method, but the possibility of making an accurate separation by this method is somewhat doubtful. It is well established that para-myosinogen is a globulin since it responds to certain of the proteid precipitation tests and is insoluble in water. Myosinogen, on the contrary, is not a typical globulin since it is soluble in water. It has been called a *pseudo-globulin*. Myosin possesses the globulin characteristics. It is insoluble in water but soluble in the other proteid solvents and is precipitated from its solution upon saturation with sodium chloride.

Under the name *extractives* we class a number of muscle constituents which occur in traces in the tissue and may be extracted by water, alcohol or ether. There are two classes of these extractives, the *non-nitrogenous extractives* and the *nitrogenous extractives*. Grouped under the non-nitrogenous bodies we have *glycogen*, *dextrin*, *sugars*, *lactic acid*, *inosit*, $C_6H_6(OH)_6$, and *fat*. In the class of nitrogenous extractives we have *creatin*, *creatinin*, *xanthin*, *hypoxanthin*, *uric acid*, *urea*, *carnin*, *phosphocarnic acid*, *inosinic acid*, *carnosin* and *taurin* (see formulas on page 210). Not all of these extractives are present in the muscles of all species of animals. Other extractives besides those enumerated above have been described and there are undoubtedly still others whose presence remains undetermined. A detailed consideration would however be unprofitable in this place.

Glycogen is an important constituent of muscle. The content of this polysaccharide in muscle varies and is markedly decreased by intense muscular activity. It is transformed into sugar and used as fuel. The liver is the organ which stores the reserve supply of glycogen and transforms it into dextrose which is passed into the blood stream and so carried to the working muscle where it is synthesized into glycogen. The glycogen thus formed is then changed into dextrose as the working muscle may need it.

Glycogen is a polysaccharide and has the same percentage composition as starch and dextrin. It resembles starch in forming an opalescent solution and resembles dextrin in being very soluble, in giving a reddish color with iodine and in being dextro-rotatory. Glycogen may be prepared from muscle by extracting with boiling water and then precipitating the glycogen from the aqueous solution by alcohol: dilute or concentrated KOH may also be used to extract the glycogen. Glycogen may be prepared in the form of a white, tasteless, amorphous powder. It is completely precipitated from its solution by saturation with solid ammonium sulphate, but is not precipitated by saturation with sodium chloride. It may also be precipitated by alcohol, tannic acid or ammoniacal basic lead acetate. It has the power of holding cupric hydroxide in solution in alkaline fluids but cannot reduce it. It may be hydrolyzed with the formation of dextrose by dilute mineral acids and is readily digested by amylolytic enzymes.

The lactic acid occurring in the muscular tissue of vertebrates is *paralactic* or *sarcoparalactic acid*,



The reaction of an inactive living muscle is alkaline, but upon the death of the muscle, or after the continued activity of a living muscle, the reaction becomes acid, due to the formation of lactic acid. There is a difference of opinion regarding the origin of this lactic acid. Some investigators claim it to arise from the carbohydrates of the muscle, while others ascribe to it a proteid origin.

Among the nitrogenous extractives of muscle, those which are of the most interest in this connection are creatin and the purin bases, xanthin and hypoxanthin. Creatin is found in varying amounts in the muscles of different species, the mus-

cles of birds having shown the largest amount. It has also been found in the blood, the brain, in transudates and in the thyroid gland. Creatin may be crystallized and forms colorless rhombic prisms (Fig. 77, below) which are soluble in warm water and practically insoluble in alcohol and ether.

FIG. 77.



CREATIN.

Upon boiling a solution of creatin with dilute hydrochloric acid it is dehydrolyzed and its anhydride creatinin is formed. The creatin of ingested meat is transformed into creatinin and excreted in the urine.

Besides being a normal constituent of muscle, xanthin has been found in the brain, spleen, pancreas, thymus, kidneys, testicles, liver, and in the urine. It may be obtained in crystalline form (Fig. 78, p. 210) but ordinarily it is amorphous. Xanthin is easily soluble in alkalis, less easily soluble in water and dilute acids, and entirely insoluble in alcohol and ether.

Hypoxanthin occurs ordinarily in those tissues and fluids which contain xanthin. It has been found, unaccompanied by xanthin, in bone marrow and in milk. Unlike xanthin it may be easily crystallized in the form of small, colorless needles.

It is readily soluble in alkalis, acids and boiling water, less soluble in cold water and practically insoluble in alcohol and ether.

The predominating inorganic salt of muscle is potassium phosphate. Besides this salt we have present sulphates, chlorides and salts of sodium, calcium, magnesium and iron.

FIG. 78.



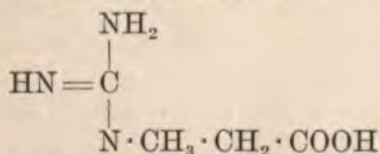
XANTHIN.

After the drawings of Horbaczewski, as represented in Neubauer and Vogel. (Ogden.)

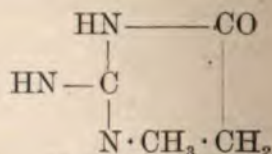
Muscular tissue is said to contain a reddish pigment called *myohæmatin*, which is a derivative of hæmoglobin.

The ordinary commercial "meat extract" is composed principally of the water-soluble constituents of muscle and *contains practically nothing of nutritive value*. The proteid material to which meat owes its value as an article of diet is practically all removed in the preparation of the extract.

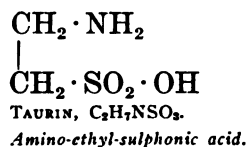
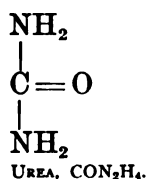
The structural formulas of the nitrogenous extractives of muscle are as follows:



CREATIN, $\text{C}_4\text{H}_9\text{N}_3\text{O}_2$.
Methyl-guanidin acetic acid.



CREATININ, $\text{C}_4\text{H}_7\text{N}_3\text{O}$.
Creatin anhydride.

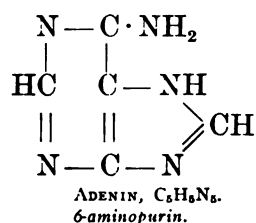
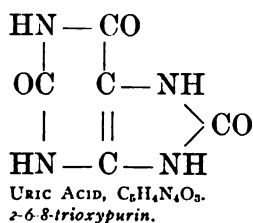
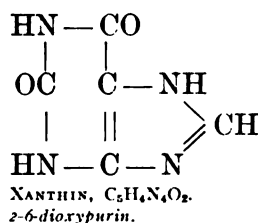
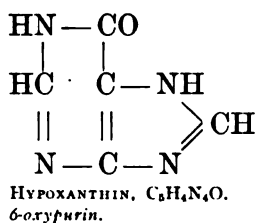
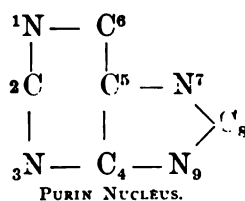
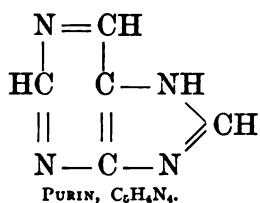


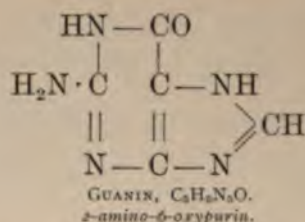
Carnosin, $\text{C}_9\text{H}_{14}\text{N}_4\text{O}_3$.

Inosinic acid, $\text{C}_{10}\text{H}_{13}\text{N}_4\text{PO}_8$.

Phosphocarnic acid, $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_5$ or $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_5$.

The following extractives as a group are called *purin bodies*. Their formulas, together with that of *purin* from which they are derived and the hypothetical "purin nucleus" follow:





EXPERIMENTS ON MUSCULAR TISSUE.

I. Experiments on "Living" Muscle.

1. **Preparation of Muscle Plasma (Halliburton).**—Wash out the blood vessels of a freshly killed rabbit with 0.9 per cent sodium chloride. This can best be done by opening the abdomen and inserting a cannula into the aorta. Now remove the skin from the lower limbs, cut away the muscles and divide them into very small pieces by means of a meat chopper. Transfer the pieces of muscle to a mortar and grind them with clean sand and a little 5 per cent magnesium sulphate. Filter off the salted muscle plasma and make the following tests:

(a) *Reaction.*—Test the reaction to litmus. What is the reaction of this fresh muscle plasma?

(b) *Fractional Coagulation.*—Place a little muscle plasma in a test-tube and arrange the apparatus for fractional coagulation as explained on page 50. Raise the temperature very carefully from 30° C. and note any changes which may occur and the exact temperature at which such changes take place. When the first proteid (para-myosinogen) coagulates filter it off and then heat the clear filtrate as before, being careful to note the exact temperature at which the next coagulation (myosinogen) occurs. There will probably be a preliminary opalescence in each case before the real coagulation occurs. Therefore do not mistake the real coagulation-point and filter at the wrong time. What are the coagulation temperatures of these two proteids? Which proteid was present in greater amount?

(c) *Formation of the Myosin Clot.*—Dilute a portion of the plasma with 3 or 4 times its volume of water and place it on a water-bath or in an incubator at 35° C. for several hours. A typical *myosin clot* should form. Note the muscle serum surrounding the clot. Now test the reaction. Has the reaction changed, and if so to what is the change due? Make a test for lactic acid. What do you conclude?

2. **Preparation of Muscle Plasma (v. Fürth).**—Remove the blood-free muscles of a rabbit as explained on page 212. Finely divide by means of a meat chopper and grind in a mortar with a little clean sand and some 0.9 per cent sodium chloride. Wrap portions of the muscle in muslin and press thoroughly by means of a tincture press or lemon squeezer. Filter and make the tests according to the directions given in the last experiment.

3. **"Fuchsin-Frog" Experiment.**—Inject a saturated aqueous solution of Fuchsin "S" into the lymph spaces of a frog three or four times daily for two or three days, in this way thoroughly saturating the tissues with the dye. Pith the animal (insert a heavy wire or blunt needle through the *occipito atlantoid* membrane), remove the skin from both hind legs and expose the sciatic nerve in one of them. Insert a small wire hook through the jaws of the frog and suspend the animal from an ordinary clamp or iron ring. Pass electrodes under the exposed sciatic nerve, and after tying the other leg to prevent any muscular movement, stimulate the exposed nerve by means of *make* and *break* shocks from an induction coil. The stimulated leg responds by pronounced muscular contractions, whereas the tied leg remains inactive. Continue the stimulation until the muscles are fatigued. The muscular activity has caused the production of *lactic acid* and this in turn has reacted with the injected fuchsin to cause a *pink or red* color to develop. The muscles of the inactive leg still remain unchanged in color.

The normal color of the Fuchsin "S" when injected was red, but upon being absorbed it became colorless through the

action of the alkalinity of the blood. Upon stimulating the muscles, however, as above explained, lactic acid was formed and this acid reacted with the fuchsin and again produced the original color of the dye.

II. Experiments on "Dead" Muscle.

1. **Preparation of Myosin.**—Take 25 grams of finely divided lean beef which has been carefully washed to remove blood and lymph constituents and place it in a beaker with 10 per cent sodium chloride. Stir occasionally for several hours. Strain off the meat pieces by means of cheese cloth, filter the solution and saturate it with sodium chloride in substance. Filter off the precipitate of *myosin* and make the tests as given below. This filtration will proceed very slowly. Myosin collects as a film on the sides of the filter paper and may be removed and tested before the entire volume of fluid has been filtered. Test the myosin precipitate as follows:

(a) *Solubility.*—Try its solubility in the ordinary solvents. Is myosin an albumin or a globulin?

(b) *Xanthoproteic Reaction.*—See page 44.

(c) *Coagulation Test.*—Suspend a little of the myosin in water in a test-tube and heat to boiling for a few moments. Now remove the suspended material and try its solubility in 10 per cent sodium chloride. What property does this experiment show myosin to possess?

Test the filtrate from the original myosin precipitate as follows:

(a) *Biuret Test.*—What does this show?

(b) Place a little of the solution in a test-tube and heat to boiling. At the boiling-point add a drop of dilute acetic acid and filter. Test this filtrate for proteose with picric acid. Is any proteose present? Saturate another portion of the filtrate with ammonium sulphate and test for peptone in the usual way (see page 59). Do you find any peptone? From your experiments on "living" and "dead" muscle what are your ideas regarding the proteids of muscle?

2. **Preparation of Glycogen.**—Grind a few scallops in a mortar with sand. Transfer to an evaporating dish, add water and boil for 20 minutes. At the boiling-point faintly acidify with acetic acid. Why is this acid added? Filter, and divide the filtrate into two parts. Note the opalescence of the solution. Test one portion of the filtrate as follows:

(a) *Iodine Test.*—To 5 c.c. of the solution in a test-tube add 2–3 drops of iodine solution and 2–3 drops of 10 per cent sodium chloride. Warm this slightly and then allow it to cool. What do you observe? Is this similar to the iodine test upon any other body with which we have had to deal?

(b) *Reduction Test.*—Does the solution reduce Fehling's solution?

(c) *Hydrolysis of Glycogen.*—Add 10 drops of concentrated hydrochloric acid to 10 c.c. of the solution and boil for 10 minutes. Cool the solution, neutralize with solid potassium hydroxide and test with Fehling's solution. Does it still fail to reduce Fehling's solution? If you find a reduction how can you prove the identity of the reducing substance?

(d) *Influence of Saliva.*—Place 5 c.c. of the solution in a test-tube, add 5 drops of saliva and place on the water-bath at 40° C. for 10 minutes. Does this now reduce Fehling's solution?

To the second part of the glycogen filtrate add 3–4 volumes of 95 per cent alcohol. Allow the glycogen precipitate to settle, decant the supernatant fluid, filter the remainder and upon the glycogen make the following tests:

(a) *Solubility.*—Try its solubility in the ordinary solvents.

(b) *Iodine Test.*—Place a small amount of the glycogen in a depression of a test-tablet and add a drop of dilute iodine solution and a trace of a sodium chloride solution. The same wine-red color is observed as in the iodine test upon the glycogen solution.

Separation of Extractives from Muscle.

1. **Creatin.**—Dissolve about 10 grams of a commercial extract of meat in 200 c.c. of warm water. Precipitate the inor-

ganic constituents by neutral lead acetate, being careful not to add an excess of the reagent. Write the equations for the reactions taking place here. Allow the precipitate to settle, then filter and remove the excess of lead in the *warm* filtrate by H_2S . Filter while the solution is yet warm, evaporate the clear filtrate to a syrup and allow it to stand at least 48 hours in a cool place. Crystals of creatin should form at this point. Examine under the microscope (Fig. 77, page 209). Treat the syrup with 200 c.c. of 88 per cent alcohol, stir well with a glass rod to bring all soluble material into solution, and then filter. The purin bases have been dissolved and are in the filtrate, whereas the creatin crystals were insoluble in the 88 per cent alcohol and remain on the filter paper. Wash the crystals with 88 per cent alcohol, then remove them and bring

FIG. 79.



HYLOXANTHIN SILVER NITRATE.

them into solution in a little hot water. Decolorize the solution by animal charcoal and concentrate it to a small volume. Allow the solution to cool and note the separation of colorless crystals of creatin. Examine these crystals under the microscope and compare them with those reproduced in Fig. 77, page 209.

2. **Hypoxanthin.**—Evaporate the alcoholic filtrate from the creatin to remove the alcohol. Make the solution ammoniacal and add ammoniacal silver nitrate until precipitation ceases. The precipitate consists principally of *hypoxanthin silver* and *xanthin silver*. Collect these silver salts on a filter paper and wash them with water. Place the precipitate and paper in an evaporating dish and boil for one minute with nitric acid having a specific gravity of 1.1. Filter while *hot* through a double paper, wash with the same strength of nitric acid and allow the solution to cool. By this treatment with nitric acid *hypoxanthin silver nitrate* and *xanthin silver nitrate* have been formed. The former is insoluble in the cold solution and separates on standing. After standing several hours filter off the hypoxanthin silver nitrate and wash with water until the wash-water is only slightly acid in reaction. Examine the crystals of *hypoxanthin silver nitrate* under the microscope and compare them with those in Fig. 79, page 216. Now wash the crystals from the paper into a beaker with a little water and warm the liquid. Remove the silver by H_2S and filter. By this means *hypoxanthin nitrate* has been formed and is present in the filtrate. Concentrate on a water-bath to drive off hydrogen sulphide and render the solution slightly alkaline with ammonia. Warm for a time, to remove the free ammonia, filter, concentrate the filtrate to a small volume and allow it to stand in a cool place. *Hypoxanthin* should crystallize in small colorless needles. Examine the crystals under the microscope.

3. **Xanthin.**—To the filtrate from the above experiment containing the *xanthin silver nitrate* add ammonia in excess. (The crystalline form of *xanthin silver nitrate* is shown in Fig. 80, p. 218.) A brownish-red precipitate of *xanthin silver* forms. Treat this suspended precipitate with H_2S (do not use an excess of H_2S), warm the mixture for a few moments and filter while hot. Concentrate the filtrate to a small volume and put away in a cool place for crystallization (Fig. 78, p. 210). To obtain xanthin in crystalline form special precautions are

generally necessary. Evaporate the solution to dryness. Make the following tests on the crystals or residue:

(a) *Xanthin Test*.—Place about one-half of the crystalline or amorphous material in a small evaporating dish, add a few

FIG. 8a.



XANTHIN SILVER NITRATE.

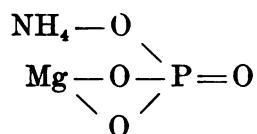
drops of concentrated nitric acid and evaporate to dryness very carefully on a water-bath. The yellow residue upon moistening with caustic potash becomes red in color and upon further heating assumes a purplish-red hue. Now add a few drops of water and warm. In this way a yellow solution results which yields a red residue upon evaporation. How does this differ from the Murexid test upon uric acid?

(b) *Weidel's Reaction*.—By gently heating bring the remainder of the xanthin crystals or residue into solution in bromine-water. Evaporate the solution to dryness on a water-bath. Remove the stopper from an ammonia bottle and by blowing across the mouth of the bottle direct the fumes of ammonia so that they come in contact with the dry residue. Under these conditions the presence of xanthin is shown by the residue assuming a red color. A somewhat brighter color may be obtained by using a trace of nitric acid with the

bromine-water. By the use of this modification however we may get a positive reaction with bodies other than xanthin.

HÜRTHLE'S EXPERIMENT.

Tease a very small piece of frog's muscle on a microscopical slide. Expose the slide to ammonia vapor for a few moments, then adjust a cover glass and examine the muscle fibers under the microscope. Note the large number of crystals of ammonium magnesium phosphate,



distributed everywhere throughout the muscle fiber, thus demonstrating the abundance of phosphates and magnesium in the muscle (Fig. 96, page 278).

CHAPTER XV.

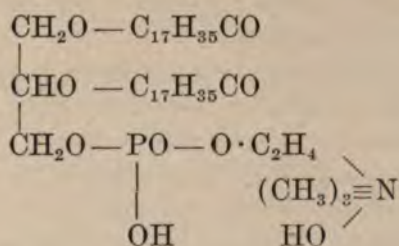
NERVOUS TISSUE.

In common with the other solid tissues of the body, nervous tissue contains a large amount of water. The percentage of water present depends upon the particular form of nervous tissue but in all forms it is invariably greater in the gray matter than in the white. Embryonic nervous tissues also contain a larger percentage of water than the tissues of adult life. The gray matter of the brain of the foetus, for instance, contains about 92 per cent of water, whereas the gray matter of the brain of the adult contains but 83-84 per cent of the fluid.

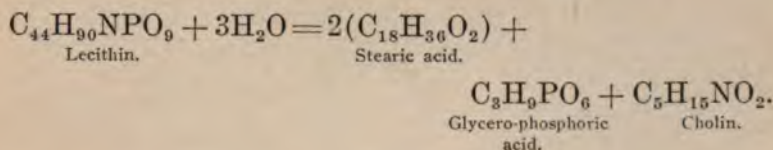
Among the solid constituents of nervous tissue are *proteids*, *cholesterin*, *cerebrin*, *lecithin*, *kephalin*, *protagon*(?), *nuclein*, *neuro-keratin*, *collagen*, *extractives* and *inorganic salts*. The proteids are present in the greatest amount and comprise about 50 per cent of the total solids. Three distinct proteids, two globulins and a nucleo-proteid, have been isolated from nervous tissue. The globulins coagulate at 47° C. and 70-75° C. respectively, while the nucleo-proteid coagulates at 56-60° C. This nucleo-proteid contains about 0.5 per cent of phosphorus (Halliburton, Levene). Nervous tissue is composed of a relatively large quantity of a variety of compounds which collectively may be grouped under the term "lipoid"—substances resembling the fats in some of their physical properties and reactions but distinct in their composition. We will class cerebrin, cholesterin and the phosphorized fats, as "lipoids."

The group of *phosphorized fats* are very important constituents of nervous tissue. The best known members of this group are *lecithin*, *protagon* (?) and *kephalin*. Lecithin occurs in larger amount than the other members of the group, has been more thoroughly studied than the others and is apparently of greater importance. Upon decomposition lecithin yields

fatty acid, *glycero-phosphoric acid* and *cholin*. Each lecithin molecule contains two fatty acid radicals which may be those of the same or different fatty acids. Thus we have different lecithins depending upon the particular fatty acid radicals which are present in the molecule. The formula of a typical lecithin would be the following:



This lecithin would be called distearyl-lecithin or *cholin-distearyl-glycero-phosphoric acid*. Upon decomposition the molecule splits according to the following reaction:



The lecithins are not confined to the nervous tissues but are found in nearly all animal and vegetable tissues. Lecithin is a primary constituent of the cell. It is soluble in chloroform, ether, alcohol, benzene and carbon disulphide. The chloroform or alcohol-ether solution may be precipitated by acetone. Lecithin may be caused to crystallize in the form of small plates by cooling the alcoholic solution to a low temperature. It has the power of combining with acids and bases, and the hydrochloric acid combination has the power of forming a double salt with platinic chloride.

Protagon, another nitrogenous phosphorized substance is a body over which there has been much discussion. Upon decomposition it is said by some investigators to yield cerebrin

and the decomposition products of lecithin. It has very recently been shown by Posner and Gies that protagon is a mixture and has no existence as a chemical individual.

Kephalin is the third member of the group of phosphorized fats. It is precipitated from its acetone-ether extract by alcohol. It contains about 4 per cent of phosphorus and has been given the formula $C_{42}H_{79}NPO_{13}$. Kephalin may be a stage in lecithin metabolism.

Cerebrin, a substance containing nitrogen but no phosphorus, is an important constituent of the white matter of nervous tissue. It has also been found in the spleen, pus and in egg yolk. It may be extracted from the tissue by boiling alcohol and is insoluble in cold alcohol, cold and hot ether and in water and dilute alkalis. Cerebrin is a mixture containing phrenosin (pseudo-cerebrin or cerebrin), a body yielding the carbohydrate galactose on decomposition.

Cholesterin, one of the primary cell constituents, is present in fairly large amount in nervous tissue. It is a mon-atomic alcohol with the formula $C_{27}H_{45}OH$. It was formerly called a "non-saponifiable fat" but since it is not changed in any way by boiling alkalis it is not a fat. It is soluble in ether, chloroform, benzene and hot alcohol. It crystallizes in the form of thin, colorless, transparent plates (Fig. 42, p. —). Cholesterin occurs abundantly in one form of biliary calculus. It has also been found in feces, wool fat, egg yolk, and milk, frequently in the form of its esters of higher fatty acids.

Nervous tissue yields about 1 per cent of ash which is made up in great part of alkaline phosphates and chlorides.

EXPERIMENTS ON THE LIPOIDS OF NERVOUS TISSUE.¹

1. **Preparation of Lecithin.**—Treat the macerated brain of a sheep with ether and allow it to stand in the cold for

¹ Preparation of So-called Protagon.—Macerate the brain of a sheep, treat with 85 per cent alcohol and warm on a water-bath at 45° C. for two hours. Filter *hot* into a bottle or strong flask and cool to 0° C. for one-half hour by means of a freezing mixture. By this procedure both protagon and cholesterin are caused to precipitate. Filter the cold solution rapidly and treat the precipitate on the paper with ice cold ether to dissolve out the cholesterin. The protagon may now be redissolved in warm 85 per cent alcohol from which solution it will precipitate upon cooling.

48-72 hours. The cold ether will extract lecithin and cholesterolin. Filter, and add acetone to the filtrate to precipitate the lecithin. Filter off the lecithin and test it as follows:

(a) *Microscopical Examination*.—Suspend a small portion in a drop of water on a slide and examine under the microscope.

(b) *Osmic Acid Test*.—Treat a small portion with osmic acid. What happens?

(c) *Acrolein Test*.—Make the acrolein test according to directions on page 100.

(d) *"Fusion" Test for Phosphorus*.—Place some of the lecithin prepared above, in a small porcelain crucible, add a suitable amount of a fusion mixture composed of KOH and KNO_3 (5:1) and heat *carefully* until the resulting mixture is colorless. Cool, dissolve the mass in a little warm water, acidify with HNO_3 , heat to boiling and add a few cubic centimeters of molybdic solution. In the presence of phosphorus a yellow precipitate forms. What is it?

2. **Preparation of Cholesterolin**.—Place a small amount of macerated brain tissue under ether and stir occasionally for one hour. Filter, exaporate the filtrate to dryness on a water-bath and test the cholesterolin according to directions given below. (If it is desired, the ether extract from the so-called protagon, or the ether-acetone filtrate from the lecithin may be used for the isolation of cholesterolin. In these cases it is simply necessary to evaporate the solution to dryness on a water-bath.) Upon the cholesterolin prepared by either of the above methods make the following tests:

(a) *Microscopical Examination*.—Examine the crystals under the microscope and compare them with those in Fig. 42, page 125.

(b) *Iodine-Sulphuric Acid Test*.—Place a few crystals of cholesterolin in one of the depressions of a test-tablet and treat with a drop of concentrated sulphuric acid and a drop of a very dilute solution of iodine. A play of colors, consisting of violet, blue, green and red, results.

(c) *The Liebermann-Burchard Test*.—Dissolve a few crystals of cholesterin in 2 c.c. of chloroform in a *dry* test-tube. Now add 10 drops of acetic anhydride and 1–3 drops of concentrated sulphuric acid. The solution becomes red, then blue, and finally bluish-green in color.

(d) *Salkowski's Test*.—Dissolve a few crystals of cholesterin in a little chloroform and add an equal volume of concentrated sulphuric acid. A play of colors from bluish-red to cherry-red and purple is noted in the chloroform, while the acid assumes a marked green fluorescence.

(e) *Schiff's Reaction*.—To a little cholesterin in an evaporating dish add a few drops of Schiff's reagent.¹ Evaporate to dryness over a low flame and observe the reddish-violet residue which changes to a bluish-violet.

(f) *Phosphorus*.—Test for phosphorus according to directions given on page 223. Is phosphorus present?

3. Preparation of Cerebrin.—Treat the macerated brain tissue, in a flask, with 95 per cent alcohol and boil on a water-bath for one-half hour, keeping the volume constant by adding fresh alcohol as needed. Filter the solution *hot* and stand the *cloudy* filtrate away for twenty-four hours. (If the filtrate is not cloudy concentrate it upon the water-bath until it is so.) Filter off the cerebrin and test it as follows:

(a) *Microscopical Examination*.—Suspend a small portion in a drop of water on a slide and examine under the microscope.

(b) *Solubility*.—Try the solubility of cerebrin in the usual solvents and in hot and cold alcohol and hot and cold ether.

(c) *Phosphorus*.—Test for phosphorus according to directions on page 223. How does the result compare with that on lecithin.

(d) Place a little cerebrin on platinum foil and warm. Note the odor.

(e) *Hydrolysis of Cerebrin*.—Place the remaining cerebrin

¹ Schiff's reagent consists of a mixture of three volumes of concentrated sulphuric acid and one volume of 10 per cent ferric chloride.

in a small evaporating dish, add equal volumes of water and dilute hydrochloric acid and boil for one hour. Cool, neutralize with *solid* potassium hydroxide, filter, and test with Fehling's solution. Is there any reduction, and if so how do you explain it?

CHAPTER XVI.

URINE: GENERAL CHARACTERISTICS OF NORMAL AND PATHOLOGICAL URINE.

Volume.—The volume of urine excreted by normal individuals, during any definite period, fluctuates within very wide limits. The average output for twenty-four hours is placed by German writers between 1,500 and 2,000 c.c. This value is not strictly applicable to conditions in America however since it has been found that the average normal excretion of the adult male American falls within the lower values of 1,000–1,200 c.c. The volume-excretion is influenced greatly by the diet, particularly by the ingestion of fluids.

Certain pathological conditions cause the output of urine for any definite period to depart very decidedly from the normal output. Among the pathological conditions in which the volume of urine is *increased* above normal are the following: Diabetes mellitus, diabetes insipidus, certain diseases of the nervous system, contracted kidney, amyloid degeneration of the kidney and in convalescence from acute diseases in general. Many drugs such as calomel, digitalis, acetates and salicylates also increase the volume of the urine excreted. A *decrease* from the normal is observed in the following pathological conditions: Acute nephritis, diseases of the heart and lungs, fevers, diarrhœa and vomiting.

Color.—Normal urine ordinarily possesses a yellow tint, the depth of the color being dependent in part upon the density of the fluid. The color of normal urine is due principally to a pigment called *urochrome*: traces of *hæmatoporphyrin*, *urobilin* and *uroerythrin*, have also been detected. Under pathological conditions the urine is subject to pronounced variations in color and may contain many varieties of pigments. Under such circumstances the urine may vary in color from an extremely light yellow to a very dark brown

or black. Vogel has constructed a color chart which is of some value for purposes of comparison. The nature and origin of the chief variations in the urinary color are set forth in tabular form by Halliburton as follows:

Color.	Cause of Coloration.	Pathological Condition.
Nearly colorless.	Dilution, or diminution of normal pigments.	Nervous conditions: hydruria, diabetes insipidus, granular kidney.
Dark yellow to brown-red.	Increase of normal, or occurrence of pathological, pigments.	Acute febrile diseases.
Milky.	Fat globules.	Chyluria.
	Pus corpuscles.	Purulent diseases of the urinary tract.
Orange.	Excreted drugs.	Santonin, chrysophanic acid.
Red or reddish.	Hæmatoporphyrin. Unchanged hæmoglobin.	Hæmorrhages, or hæmoglobinuria.
	Pigments in food (log-wood, madder, bilberries, fuchsin).	
Brown to brown-black.	Hæmatin.	Small hæmorrhages.
	Methæmoglobin.	Methæmoglobinuria.
	Melanin.	Melanotic sarcoma.
	Hydrochinon and catechol.	Carbolic-acid poisoning.
Greenish-yellow, greenish-brown, approaching black.	Bile-pigments.	Jaundice.
Dirty green ¹ or blue.	A dark-blue scum on surface, with a blue deposit, due to an excess of indigo-forming substances.	Cholera, typhus; seen especially when the urine is putrefying.
Brown-yellow to red-brown, becoming blood-red upon adding alkalis.	Substances contained in senna, rhubarb, and chelidonium which are introduced into the system.	

¹ This dirty green or blue color also occurs after the use of methylene-blue in the organism.

Transparency.—Normal urine is ordinarily perfectly clear and transparent when voided. On standing for a variable time, however, a cloud (nubecula) consisting principally of nucleo-proteid or mucoid (see p. 264) and epithelial cells forms. A turbidity due to the precipitation of phosphates is normally noted in urine passed after a hearty meal. The urine obtained 2–3 hours after a meal or later is ordinarily free from turbidity. Permanently turbid urines ordinarily arise from pathological conditions.

Odor.—The odor of normal urine is of a faint, aromatic type. The bodies to which this odor is due are not well known, but it is claimed by some investigators to be due, at least in part, to the presence of minute amounts of certain volatile organic acids. When the urine undergoes decomposition, *e. g.*, in alkaline fermentation a very unpleasant ammoniacal odor is evolved. All urines are subject to such decomposition if allowed to stand for a sufficiently long time. Under normal conditions the urine very often possesses a peculiar odor due to the ingestion of some certain drug or vegetable. For instance, cubebs, copaiba, myrtol, saffron, tolu and turpentine each imparts a somewhat specific odor to the urine. After the ingestion of asparagus, the urine also possesses a typical odor.

Frequency of Urination.—The frequency of urination varies greatly in different individuals but in general is dependent upon the amount of fluid in the bladder. In pathological conditions an inflammatory affection of the urinary tract or any disturbance of the innervation of the bladder will influence the frequency. Affections of the spinal cord which lead to an increased irritability of the bladder or a weakening of the sphincter will result in increasing the frequency of urination.

Reaction.—The mixed twenty-four hour urinary excretion of a normal individual ordinarily possesses an acid reaction to litmus. This acidity is now believed to be due to the presence of various acidic radicals and not to the presence of

sodium di-hydrogen phosphate as was formerly held (see Phosphates, p. 275). The acidity imparted to the urine by any particular acid depends entirely upon the extent to which

FIG. 81.



DEPOSIT IN AMMONIACAL FERMENTATION.

a, Acid ammonium urate; *b*, ammonium magnesium phosphate; *c*, bacteria.

FIG. 82.



DEPOSIT IN ACID FERMENTATION.

a, Fungus; *b*, amorphous sodium urate; *c*, uric acid; *d*, calcium oxalate.

the acid is dissociable, since it is the hydrogen ion which is responsible for the acid reaction.

The composition of the food is perhaps the most important

factor in determining the reaction of the urine. The reaction ordinarily varies considerably according to the time of day the urine is passed. For instance for a variable length of time after a meal the urine may be neutral or even alkaline in reaction to litmus, owing to the claim of the gastric juice upon the acidic radicals to further the formation of hydrochloric acid for use in carrying out the digestive secretory function. This change in reaction is known as the *alkaline tide* and is common to perfectly healthy individuals. The urine may also become temporarily alkaline in reaction to litmus, as the result of ingesting alkaline carbonates or certain salts of tartaric and citric acids which may be transformed into carbonates within the organism. Normal urine upon standing for some time becomes alkaline in reaction to litmus, owing to the inception of alkaline or ammoniacal fermentation through the agency of micro-organisms. This fermentation has no especial diagnostic value except in cases where the urine has undergone this change *within the organism* and is voided in the decomposed state. Ammoniacal fermentation is ordinarily due to cystitis or occurs as the result of infection in the process of catheterization. A microscopical examination of such urine (Fig. 81, p. 229) shows the presence of *ammonium magnesium phosphate* crystals, *amorphous phosphates* and not infrequently *ammonium urate*.

Occasionally a urine which possesses a normal acidity when voided, upon standing instead of undergoing ammoniacal fermentation as above described will become still more strongly acid in reaction. Such a phenomenon is termed *acid fermentation*. Accompanying this increased acidity there is ordinarily a deepening of the tint of the urinary color. Such urines may contain *acid urates*, *uric acid*, *fungi* and *calcium oxalate* (Fig. 82, p. 229). On standing for a sufficiently long time any urine which exhibits acid fermentation will ultimately change in reaction, due to the inception of alkaline fermentation, and will show the microscopical deposits characteristic of such a urine.

Specific Gravity.—The specific gravity of the urine of normal individuals varies ordinarily between 1.015 and 1.025.

This value is subject to wide fluctuations under various conditions. For instance following copious water- or beer-drinking the specific gravity may fall to 1.003 or lower, whereas in cases of excessive perspiration it may rise as high as 1.040 or even higher. Where a very accurate determination of the specific gravity is desired use is commonly made of the *pyknometer* or of the *Westphal hydrostatic balance*. These instruments, however, are not suited for clinical use. The clinical method of determining the specific gravity is by means of a *urinometer* (Fig. 83, p. 231). This affords a very rapid method and at the same time is sufficiently accurate for clinical purposes. The urinometer is always calibrated for use at a specific temperature and the observations made at any other temperature must be subjected to a certain correction to obtain the true specific gravity. In making this correction *one unit of the last order is added* to the observed specific gravity for every three degrees *above* the normal temperature and *subtracted* for every three degrees *below* the normal temperature. For instance, if in using a urinometer calibrated for 15° C. the specific gravity of a urine having a temperature of 21° C. is determined as 1.018 it is necessary to add to the observed specific gravity two units of the third order to obtain the real specific gravity of the urine. Therefore the true specific gravity, at 15° C., of a urine having a specific gravity of 1.018 at 21° C. is $1.018 + 0.002 = 1.020$.

Pathologically, the specific gravity may be subjected to very wide variations. This is especially true in diseases of the kidneys. In acute nephritis ordinarily the urine is concentrated and of a high specific gravity whereas in chronic nephritis the

FIG. 83.



URINOMETER AND CYLINDER.

reverse conditions are more apt to prevail. In fact under most conditions, whether physiological or pathological, the specific gravity of the urine is inversely proportional to the volume excreted. This is not true of diabetes mellitus, however, where the volume of urine is large and the specific gravity is also high, owing to the sugar contained in the urine.

The amount of solids eliminated in the excretion for twenty-four hours may be roughly calculated by means of *Long's Coefficient*, i. e., 2.6. The solid content of 1,000 c.c. of urine is obtained by multiplying the last two figures of the specific gravity observed at 25° C. by 2.6. To determine the amount of solids excreted in twenty-four hours if the volume was 1,120 c.c. and the specific gravity was 1.018 the calculation would be as follows:

(a) $18 \times 2.6 = 46.8$ grams of solid matter in 1,000 c.c. of urine.

(b) $\frac{46.8 \times 1120}{1000} = 52.4$ grams of solid matter in 1,120 c.c. of urine.

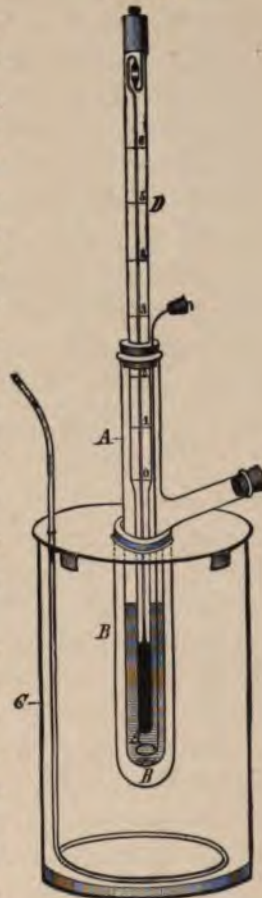
The coefficient of Häser (2.33) which has been in use for years probably gives values that are inaccurate for conditions existing in America.

Freezing-Point (Cryoscopy).—The freezing-point of a solution depends upon the total number of molecules of solid matter dissolved in it. The determination of the osmotic pressure by this method has recently come to be of some clinical importance particularly as an aid in the diagnosis of kidney disorders. In this connection it is best to collect the urine from each kidney separately and determine the freezing-point in the individual samples so collected. By this means considerable aid in the diagnosis of renal diseases may be secured. The fluids most frequently examined cryoscopically are the blood (see p. 148) and the urine. The freezing-point is denoted by Δ . The value of Δ for normal urine varies ordinarily between -1.3° and -2.3° C., the freezing-point of pure water being taken as 0° . Δ is subject to very wide fluc-

tuations under unusual conditions. For instance following copious water- or beer-drinking Δ may have as high a value as -0.2° C. whereas on a diet containing much salt and deficient in fluids the value of Δ may be lowered to -3° C. or even lower. The freezing-point of normal blood is generally about -0.56° C. and it not subject to the wide variations noted in the urine, because of the tendency of the organism to maintain the normal osmotic pressure of the blood under all conditions. Variations between -0.51° and -0.62° C. may be due entirely to dietary conditions but if any marked variation is noted it can, in most cases, be traced to a disordered kidney function.

Freezing-point determinations may be made by means of the Beckmann-Heidenhain apparatus (Fig. 84, p. 233) or the Zikel Pektoscope. The Beckmann-Heidenhain apparatus consists of the following parts: A strong battery jar or beaker (C) furnished with a metal cover which is provided with a circular hole in its center. This strong glass vessel serves to hold the freezing mixture by means of which the temperature of the fluid under examination is lowered. A large glass tube (B) designed as an air-jacket, and formed after the manner of a test-tube is introduced through the central aperture in the metal cover

FIG. 84.



BECKMANN-HEIDENHAIN
FREEZING-POINT AP-
PARATUS. (Long.)

D, a delicate thermometer; *C*, the containing jar; *B*, the outside or air mantle tube; *A*, the tube in which the mixture to be observed is placed. Two stirrers are shown, one for the cooling mixture in the jar and one for the experimental mixture.

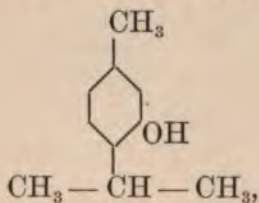
and into this air-jacket is lowered a smaller tube (A) containing the fluid to be tested. A very delicate thermometer (D), graduated in hundredths of a degree is introduced into the inner tube and is held in place by means of a cork so that the mercury bulb is immersed in the fluid under examination but does not come in contact with any glass surface. A small platinum wire stirrer serves to keep the fluid under examination well mixed while a larger stirrer is used to manipulate the freezing mixture. (Rock salt and ice in the proportion 1:3 form a very satisfactory freezing mixture.)

In making a determination of the freezing-point of a fluid by means of the Beckmann-Heidenhain apparatus proceed as follows: Place the freezing mixture in the battery jar and add water (if necessary) to secure a temperature not lower than 3° C. Introduce the fluid to be tested into tube A, place the thermometer and platinum wire stirrer in position and insert the tube into the air jacket which has previously been inserted through the metal cover of the battery jar. Manipulate the two stirrers in order to insure an equalization of temperature and observe the course of the mercury column of the thermometer very carefully. The mercury will *gradually fall* and this gradual lowering of the temperature will be followed by a *sudden rise*. The point at which the mercury rests *after this sudden rise* is the *freezing-point*. This rise is due to the fact that previous to freezing, a fluid is always more or less *over cooled* and the thermometer temporarily registers a temperature somewhat *below the freezing-point*. As the fluid freezes however there is a very *sudden* change in the temperature of the liquid and this change is imparted to the thermometer and causes the rise as indicated. It occasionally occurs that the fluid under examination is very much over cooled and *does not freeze*. Under such circumstances a small piece of ice is introduced into it by means of the side tube noted in the figure. This so-called "inoculation" causes the fluid to freeze instantaneously. (For details of the method

of determining the freezing-point consult standard works on physical or organic chemistry.)

Electrical Conductivity.—The electrical conductivity of the urine is dependent upon the number of *inorganic* molecules or ions present, and in this differs from the freezing-point which is dependent upon the total number of molecules both *inorganic* and *organic* which are in solution. The conductivity of the urine has been investigated but slightly, and this very recently, but from the data secured it seems that the value generally falls below $\kappa = 0.03$. The conductivity of blood serum has been determined as $\kappa = 0.012$. Up to the present time the determination of the electrical conductivity of any of the fluids of the body has been put to very slight clinical use. Experience may show the conductivity value to be a more important aid to diagnosis than it is now considered particularly if it is taken in connection with the determination of the freezing-point. By a combination of these two methods the portion of the osmotic pressure due respectively to electrolytes and non-electrolytes may be determined. For a discussion of electrical conductivity, the method by which it is determined and the principles involved consult standard works on physical or electrochemistry.

Collection of the Urine Sample.—If any dependable data are desired regarding the *quantitative* composition of the urine the examination of the mixed excretion for twenty-four hours is *absolutely necessary*. In collecting the urine the bladder may be emptied at a given hour, say 8 A. M., the urine discarded and all the urine from that hour up to and including that passed the next day at 8 A. M. saved, thoroughly mixed and a sample taken for analysis. Powdered thymol,



is a very satisfactory preservative since the excess may be removed by filtration, if desired, and any small amount which may go into solution will have no appreciable influence upon the determination of any of the urinary constituents. It has no reducing power and so may safely be used to preserve diabetic urines. To insure the preservation of the mixed urine of the twenty-four hour period it is advisable to place a small amount of the thymol powder in the urine receptacle before the first fraction of urine is voided.

In certain pathological conditions it is desirable to collect the urine passed during the *day* separately from that passed during the *night*. When this is done the urine voided between 8 A. M. and 8 P. M. may be taken as the *day sample* and that voided between 8 P. M. and 8 A. M. as the *night sample*.

The *qualitative* testing of urine voided at *random*, except in a few specific instances, is of no particular value so far as giving us any accurate knowledge as to the exact urinary characteristics of the individual is concerned. In the great majority of cases the qualitative as well as the quantitative tests should be made upon the mixed excretion for a twenty-four hour period.

CHAPTER XVII.

URINE: PHYSIOLOGICAL CONSTITUENTS.¹

1. Organic Physiological Constituents.

Urea.

Uric acid.

Creatinin.

Ethereal sulphuric acids...	{	Phenol- and <i>p</i> -cresol-sulphuric acids.
		Pyrocatechin-sulphuric acid.
		Indoxyl-sulphuric acid.
		Skatoxyl-sulphuric acid.

Hippuric acid.

Oxalic acid.

Neutral sulphur compounds.	{	Cystin.
		Chondroitin-sulphuric acid.
		Sulphocyanides.
		Taurin derivatives.
		Oxyproteic acid.
		Alloxyproteic acid.
		Uroferric acid.

Allantoïn.

Aromatic oxyacids.....	{	Paraoxyphenyl-acetic acid.
		Paraoxyphenyl-propionic acid.
		Homogentisic acid.
		Uroleucic acid.
		Oxymandelic acid.
		Kynurenic acid.

Benzoic acid.

Nucleo-proteid.

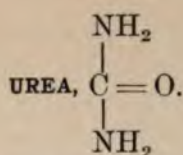
Oxaluric acid.

¹ It is impossible to make any *absolute* classification of the physiological and pathological constituents of the urine. A substance may be present in the urine in small amount physiologically and be sufficiently increased under certain conditions as to be termed a pathological constituent. Therefore it depends, in some instances, upon the *quantity* of a constituent present whether it may be correctly termed a physiological or a pathological constituent.

Enzymes	{ Pepsin. Diastatic enzyme (Amylase).
Volatile fatty acids.....	{ Acetic acid. Butyric acid. Formic acid.
Paralactic acid.	
Phenaceturic acid.	
Phosphorized compounds...	{ Glycerophosphoric acid. Phosphocarnic acid.
Pigments	{ Urochrome. Urobilin. Uroerythrin.
Ptomaines and leucomaines.	
Purin bases.....	{ Adenin. Guanin. Xanthin. Epiguanin. Episarkin. Hypoxanthin. Paraxanthin. Heteroxanthin. 1-Methylxanthin.

2. Inorganic Physiological Constituents.

Ammonia.
 Sulphates.
 Chlorides.
 Phosphates.
 Sodium and potassium.
 Calcium and magnesium.
 Carbonates.
 Iron.
 Fluorides.
 Nitrates.
 Silicates.
 Hydrogen peroxide.



Urea is the principal end-product of the metabolism of proteid bodies. It has been generally believed that about 90 per cent of the total nitrogen of the urine was present as urea. Recently, however, Folin has shown that the distribution of the nitrogen of the urine among urea and the other nitrogen-containing bodies present depends entirely upon the absolute amount of the total nitrogen excreted. He found that a decrease in the total nitrogen excretion was always accompanied by a decrease in the percentage of the total nitrogen excreted as urea, and that after so regulating the diet of a normal per-

FIG. 85.



UREA.

son as to cause the excretion of total nitrogen to be reduced to 3-4 grams in 24 hours, *only about 60 per cent of this nitrogen appeared in the urine as urea.* His experiments also seem to show urea to be the only one of the nitrogenous excretions which is relatively as well as absolutely decreased as a result

of decreasing the amount of proteid metabolized. This same investigator reports a hospital case in which only 14.7 per cent of the total nitrogen was present as urea and about 40 per cent was present as ammonia. Mörner had previously reported a case in which but 4.4 per cent of the total nitrogen of the urine was present as urea, and 26.7 per cent was present as ammonia.

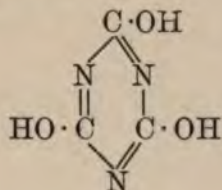
Urea occurs most abundantly in the urine of man and carnivora and in somewhat smaller amount in the urine of herbivora; the urine of fishes, amphibians and certain birds also contains a small amount of the substance. Urea is also found in nearly all the fluids and in many of the tissues and organs of mammals. The amount excreted under normal conditions, by an adult man in 24 hours is about 30 grams; women excrete a somewhat smaller amount. The excretion is greatest in amount after a diet of meat, and least in amount after a diet consisting of non-nitrogenous foods; this is due to the fact that the last mentioned diet has a tendency to decrease the metabolism of the tissue proteids and thus cause the output of urea under these conditions to fall below the output of urea observed during starvation. The output of urea is also increased after copious water- or beer-drinking. This increase is due *primarily* to the washing out of the tissues of the urea previously formed, but which had not been removed in the normal processes, and, *secondarily* to a stimulation of proteid catabolism.

Urea may be formed in the organism from amino acids such as leucin, glycocoll and aspartic acid: it may also be formed from ammonium carbonate $(\text{NH}_4)_2\text{CO}_3$ or ammonium carbamate, $\text{H}_4\text{N} \cdot \text{O} \cdot \text{CO} \cdot \text{NH}_2$.

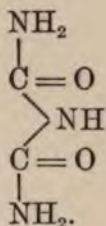
There are differences of opinion regarding the transformation of the substances just named into urea but there is rather conclusive evidence that at least a part of the urea is formed in the liver; it may be formed in other organs or tissues as well.

Urea crystallizes in long, colorless, four or six-sided, anhydrous, rhombic prisms (Fig. 85, p. 239), which melt at 132°C . and are soluble in water or alcohol and insoluble in ether or

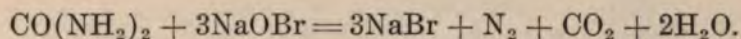
chloroform. If a crystal of urea is heated in a test-tube, it melts and decomposes with the liberation of ammonia. The residue contains *cyanuric acid*,



and *biuret*,



The biuret may be dissolved in water and a reddish-violet color obtained by treating the aqueous solution with cupric sulphate and potassium hydroxide (see Biuret Test, p. 45). Certain hypochlorites or hypobromites in alkaline solution have the power of decomposing urea into nitrogen, carbon dioxide and water. Sodium hypobromite brings about this decomposition as follows:



This property forms the basis for the clinical quantitative determination of urea (see page 351).

Urea has the power of forming crystalline compounds with certain acids: urea nitrate and urea oxalate are the most important of these compounds. *Urea nitrate*, $\text{CO}(\text{NH}_2)_2 \cdot \text{HNO}_3$, crystallizes in colorless, rhombic or six-sided tiles (Fig. 86, p. 242), which are easily soluble in water. *Urea oxalate*, $2 \cdot \text{CO}(\text{NH}_2)_2 \cdot \text{H}_2\text{C}_2\text{O}_4$, crystallizes in the form of rhombic or six-sided prisms or plates (Fig. 88, p. 244): the oxalate differs from the nitrate in being somewhat less soluble in water.

A decrease in the excretion of urea is observed in many diseases in which the diet is much reduced and in some disorders as a result of alterations in metabolism, *e. g.*, myxœdema, and in others as a result of changes in excretion, as in

FIG. 86.



UREA NITRATE.

severe and advanced kidney disease. A pathological increase is found in a large proportion of diseases which are associated with a toxic state.

EXPERIMENTS ON UREA.

1. **Isolation from the Urine.**—Place 800 c.c. of urine in a precipitating jar, add 250 c.c. of baryta mixture¹ and stir thoroughly. Filter off the precipitate of phosphates, sulphates, urates and hippurates and evaporate the filtrate on a water-bath to a thick syrup. This syrup contains chlorides, creatinin, organic salts, pigments and urea. Extract the syrup with warm 95 per cent alcohol and filter again. The filtrate contains the urea contaminated with pigment. Decolorize the filtrate by boiling with animal charcoal, filter again and stand the

¹ Baryta mixture consists of a mixture of one volume of a saturated solution of $\text{Ba}(\text{NO}_3)_2$ and two volumes of a saturated solution of $\text{Ba}(\text{OH})_2$.

filtrate away in a cold place for crystallization. Examine the crystals under the microscope and compare them with those shown in Fig. 85, page 239.

2. **Solubility.**—Test the solubility of urea, prepared by yourself or furnished by the instructor, in the ordinary solvents (see p. 4) and in alcohol and ether.

3. **Melting-Point.**—Determine the melting-point of some pure urea furnished by the instructor. Proceed as follows: Into an ordinary melting-point tube, sealed at one end, introduce a crystal of urea. Fasten the tube to the bulb of a thermometer as shown in Fig. 87, p. 243, and suspend the bulb and its attached tube in a small beaker containing sulphuric acid. Gently raise the temperature of the acid by means of a low flame, stirring the fluid continually, and note the temperature at which the urea begins to melt.

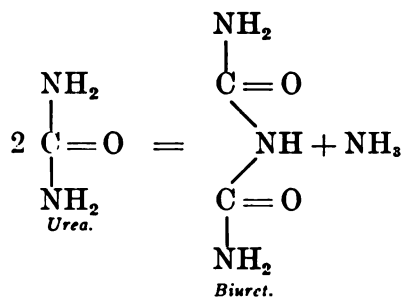
4. **Crystalline Form.**—Dissolve a crystal of pure urea in a few drops of 95 per cent alcohol and place 1–2 drops of the alcoholic solution on a microscopic slide. Allow the alcohol to evaporate spontaneously, examine the crystals under the microscope and compare them with those reproduced in Fig. 85, p. 239. Recrystallize a little urea from water in the same way and compare the crystals with those obtained from the alcoholic solution.

5. **Formation of Biuret.**—Place a small amount of urea in a *dry* test-tube and heat carefully in a low flame. The urea melts at 132° C. and liberates ammonia. Continue heating until the fused mass begins to solidify. Cool the tube, dissolve the residue in dilute potassium hydroxide solution and add very dilute cupric sulphate solution (see p. 45). The

FIG. 87.

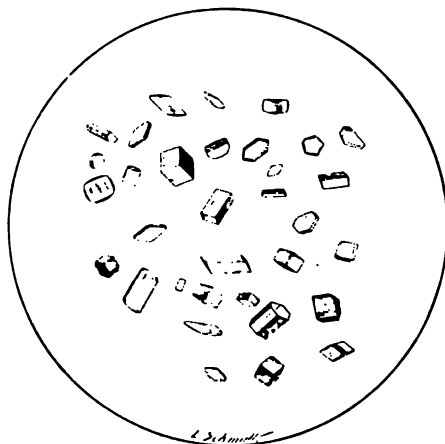
MELTING-POINT TUBES,
FASTENED TO BULB OF
THERMOMETER.

purplish-violet color is due to the presence of biuret which has been formed from the urea through the application of heat as indicated. This is the reaction:



6. **Urea Nitrate.**—Prepare a concentrated solution of urea by dissolving a little of the substance in a few drops of water. Place a drop of this solution on a microscopic slide, add a drop

FIG. 88.



UREA OXALATE.

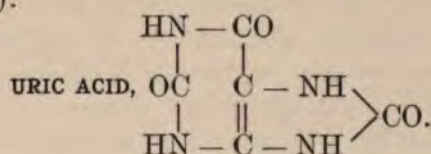
of concentrated nitric acid and examine under the microscope. Compare the crystals with those reproduced in Fig. 86, p. 242.

7. **Urea Oxalate.**—To a drop of a concentrated solution of urea, prepared as described in the last experiment (6), add a drop

of a saturated solution of oxalic acid. Examine under the microscope and compare the crystals with those shown in Fig. 88, page 244.

8. **Decomposition by Sodium-Hypobromite.**—Into a mixture of 3 c.c. of concentrated sodium hydroxide solution and 2 c.c. of bromine water in a test-tube introduce a crystal of urea or a small amount of a concentrated solution of urea. Through the influence of the sodium-hypobromite, NaOBr, the urea is decomposed and carbon dioxide and nitrogen are liberated. The carbon dioxide is absorbed by the excess of sodium hydroxide while the nitrogen is evolved and causes the marked effervescence observed. This property forms the basis for one of the methods in common use for the quantitative determination of urea. Write the equation showing the decomposition of urea by sodium-hypobromite.

9. **Furfurol Test.**—To a few crystals of urea in a small porcelain dish add 1–2 drops of a concentrated aqueous solution of furfural and 1–2 drops of concentrated hydrochloric acid. Note the appearance of a yellow color which gradually changes into a purple. Allantoin also responds to this test (see page 261).



Uric acid is one of the most important of the constituents of the urine. Normally about 0.7 gram is excreted in 24 hours but this amount is subject to wide variations, particularly under certain dietary and pathological conditions. Uric acid is a diureide and consequently upon oxidation yields two molecules of urea. It acts as a weak dibasic acid and forms two classes of salts, neutral and acid. The neutral potassium and lithium urates are the most easily soluble of the alkali salts; the ammonium urate is difficultly soluble. The acid-alkali urates are more insoluble and form the major portion of the sediment

which separates upon cooling concentrated urine; the alkaline earth urates are very insoluble. Ordinarily uric acid occurs in the urine in the form of urates and upon acidifying the liquid the uric acid is liberated and deposits in crystalline form. This property forms the basis for one of the older methods for the quantitative determination of uric acid (Heintz Method, p. 350).

Uric acid is very closely related to the purin bases as may be seen from a comparison of its structural formula with those of the purin bases given on page 211. According to the purin nomenclature it is designated 2-6-8-trioxypurin. Uric acid forms the principal end-product of the nitrogenous metabolism of birds and scaly amphibians; in the human organism it occupies the fourth position inasmuch as here urea, ammonia and creatinin are the chief end-products of nitrogenous metabolism. The relation existing between uric acid and urea in human urine under normal conditions varies on the average from 1:40 to 1:100 and is subject to wider variations under pathological conditions. Because of the high content of uric acid in the urine of new-born infants the ratio may be reduced to 1:10 or even lower.

In man, uric acid probably results principally from the destruction of nuclein material. It may arise from nuclein or other purin material ingested as food or from the disintegrating cellular matter of the organism. The uric acid resulting from the first process is said to be of *exogenous* origin, whereas the product of the second form of activity is said to be of *endogenous* origin. As the result of experimentation, Sivé, and Burian and Schur, and Rockwood claim that the amount of endogenous uric acid formed in any given period is fairly constant for each individual under normal conditions, and that it is entirely independent of the total amount of nitrogen eliminated. Recently Folin has taken exception to the statements of these investigators and claims that, following a pronounced decrease in the amount of proteid metabolized, the absolute quantity of uric acid is decreased but that this decrease is relatively smaller than the decrease

PLATE V.



URIC ACID CRYSTALS. NORMAL COLOR. (From Purdy, after Peyer.)

in the total nitrogen excretion and that the per cent of the uric acid nitrogen, in terms of the total nitrogen, is therefore decidedly increased.

In birds and scaly amphibians the formation of uric acid is analogous to the formation of urea in man. In these organisms it is derived principally from the proteid material of the tissues and the food and is formed through a process of synthesis which occurs for the most part in the liver; a comparatively small fraction of the total uric acid excretion of birds and scaly amphibians may result from nuclein material.

When pure, uric acid may be obtained as a white, odorless, and tasteless powder which is composed principally of small transparent crystalline rhombic plates. Uric acid as it separates from the urine is invariably pigmented, and crystallizes in a large variety of characteristic forms, *e. g.*, dumb-bells, wedges, rhombic prisms, irregular rectangular or hexagonal plates, whetstones, prismatic rosettes, etc. Uric acid is insoluble in alcohol and ether, soluble with difficulty in boiling water (1:1800) and practically insoluble in cold water (1:39,480, at 18° C.). It is soluble in alkalis, alkali carbonates, boiling glycerin, concentrated sulphuric acid and in certain organic bases such as ethylamine and piperidin. It is claimed that the uric acid is held in solution in the urine by the urea and di-sodium hydrogen phosphate present. Uric acid possesses the power of reducing cupric hydroxide in alkaline solution and may thus lead to an erroneous conclusion in testing for sugar in the urine by means of Fehling's or Trommer's tests. A white precipitate of cuprous urate is formed if only a small amount of cupric hydroxide is present, but if enough of the copper salt is present the characteristic red or brownish-red precipitate of cuprous oxide is obtained. Uric acid does not possess the power of reducing bismuth in alkaline solution and therefore does not interfere in testing for sugar in the urine by means of Boettger's or Nylander's tests.

In addition to being an important urinary constituent uric acid is normally present in the brain, heart, liver, lungs, pan-

creas and spleen; it also occurs in the blood of birds and has been detected in traces in human blood under normal conditions.

Pathologically, the excretion of uric acid is subject to wide variations but the experimental findings are rather contradictory. It may be stated with certainty, however, that in leukæmia the uric acid output is increased absolutely as well as relatively to the urea output; under these conditions the ratio between the uric acid and urea may be as low as 1:9, whereas the normal ratio, as we have seen, is 1:50 or higher. In the study of the influence of X-ray on metabolism Edsall has very recently reached some interesting conclusions. He found that the excretion of uric acid is usually increased and that in some conditions, particularly in leukæmia, it may be *greatly* increased. The excretion of total nitrogen, phosphates and other substances may also be considerably increased.

EXPERIMENTS ON URIC ACID.

1. **Isolation from the Urine.**—Place about 200 c.c. of filtered urine in a beaker, render it acid with 2–10 c.c. of concentrated hydrochloric acid, stir thoroughly and stand the vessel in a cold place for 24 hours. Examine the pigmented crystals of uric acid under the microscope and compare them with those shown in Fig. 101, p. 323 and Pl. V. opposite p. 247.

2. **Solubility.**—Try the solubility of pure uric acid, furnished by the instructor, in the ordinary solvents (see p. 4) and in alcohol, ether, concentrated sulphuric acid and in boiling glycerin.

3. **Crystalline Form of Pure Uric Acid.**—Place about 100 c.c. of water in a small beaker, render it distinctly alkaline with potassium hydroxide solution and add a small amount of pure uric acid stirring continuously. Cool the solution, render it distinctly acid with hydrochloric acid and allow it to stand in a cool place for crystallization. Examine the crystals under the microscope and compare them with those reproduced in Fig. 89, page 249.

4. **Murexid Test.**—To a small amount of pure uric acid in a small evaporating dish add 2–3 drops of concentrated nitric acid. Evaporate to dryness carefully on a water-bath or over a very low flame. A red or yellow residue remains

FIG. 89.



PURE URIC ACID.

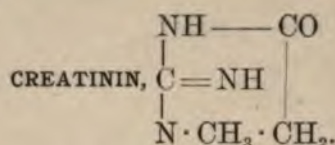
which turns purplish-red after cooling the dish and adding a drop of very dilute ammonium hydroxide. The color is due to the formation of *murexid*. If potassium hydroxide is used instead of ammonium hydroxide a purplish-violet color due to the production of the potassium salt is obtained. The color disappears upon warming; with certain related bodies (purin bases) the color persists under these conditions.

5. **Schiff's Reaction.**—Dissolve a small amount of pure uric acid in sodium carbonate solution and transfer a drop of the resulting mixture to a strip of filter paper saturated with argentic nitrate solution. A yellowish-brown or black coloration due to the formation of reduced silver is produced.

6. **Influence upon Fehling's Solution.**—Dilute 1 c.c. of Fehling's solution with 4 c.c. of water and heat to boiling. Now add *slowly*, a few drops at a time, 1–2 c.c. of a concentrated solution of uric acid in potassium hydroxide, heating

after each addition. From this experiment what do you conclude regarding the possibility of arriving at an erroneous decision when testing for sugar in the urine by means of Fehling's test?

7. **Reduction of Nylander's Reagent.**—To 5 c.c. of a solution of uric acid in potassium hydroxide add about one-half a cubic centimeter of Nylander's reagent and heat to boiling for a few moments. Do you obtain the typical black end-reaction signifying the reduction of the bismuth?



Creatinin is the anhydride of creatin and is a constituent of normal human urine. It is derived from the creatin of ingested muscular tissue as well as from the creatin of the muscular tissue of the organism. Under normal conditions about 1 gram of creatinin is excreted by an adult man in 24 hours, the exact amount depending in great part upon the nature of the food and decreasing markedly in starvation. Very little that is important is known regarding the excretion of creatinin under pathological conditions. The creatinin content of the urine is said to be increased in typhoid fever, typhus, tetanus and pneumonia, and to be decreased in anæmia, chlorosis, paralysis and in advanced degeneration of the kidneys. The greater part of the data, however, relating to the variation of the creatinin excretion under pathological conditions are not of much value since, in nearly every instance, the diet was not sufficiently controlled to permit the collection of reliable data.

Creatinin crystallizes in colorless, glistening monoclinic prisms (Fig. 90, p. 251) which are soluble in about 12 parts of cold water; they are more soluble in warm water and in warm alcohol. One of the most important and interesting of the compounds of creatinin is *creatinin-zinc chloride*,

$(C_4H_7N_3O)_2ZnCl_2$, which is formed from an alcoholic solution of creatinin upon treatment with zinc chloride in acid solution. Creatinin has the power of reducing cupric hydroxide in alkaline solution and in this way may interfere with the determination of sugar in the urine. In the reduction by creatinin the blue liquid is first changed to a yellow and the formation of a brownish-red precipitate of cuprous oxide is brought about only after continuous boiling with an excess of the copper salt. Creatinin does not reduce alkaline bismuth solutions and therefore does not interfere with Nylander's and Boettger's tests.

FIG. 90.



CREATININ.

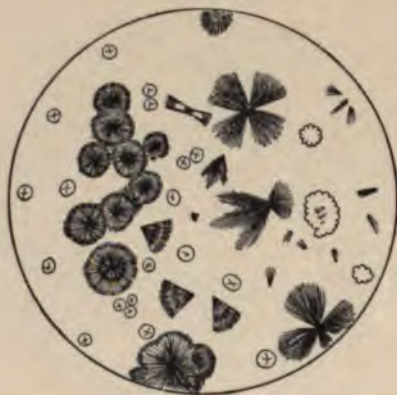
It has very recently been shown by Folin that the absolute quantity of creatinin eliminated in the urine on a meat-free diet is a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated.

EXPERIMENTS ON CREATININ.

✓ 1. **Separation from the Urine.**—Place 250 c.c. of urine in a casserole or beaker, render it alkaline with milk of lime and then add $CaCl_2$ solution until the phosphates are completely

precipitated. Filter off the precipitate, render the filtrate slightly acid with acetic acid and evaporate it to a syrup. While still warm this syrup is treated with about 50 c.c. of 95-97 per cent alcohol and the mixture allowed to stand 8-12 hours in a cool place. The precipitate is now filtered off and the filtrate treated with a little sodium acetate and about one-half c.c. of acid-free zinc chloride solution having a specific gravity of 1.2. This mixture is stirred thoroughly and allowed to stand in a cold place for 48-72 hours. Creatinin-zinc chloride (Fig. 91, below) will crystallize out under these con-

FIG. 91.



CREATININ-ZINC CHLORIDE. (Salkowski.)

ditions. Collect the crystals on a filter paper and wash them with alcohol to remove chlorides. Now treat the zinc chloride compound with a little warm water, boil with lead oxide and filter. The filtrate may now be decolorized by animal charcoal, evaporated to dryness and the residue extracted with strong alcohol. (Creatin remains undissolved under these conditions.) The alcoholic extract of creatinin is now evaporated to incipient crystallization and left in a cool place until crystallization is complete. If desired the crystals may be purified by recrystallization from water.

2. **Weyl's Test.**—Take 5 c.c. of urine in a test-tube, add a

few drops of sodium nitro-prusside and render the solution alkaline with potassium hydroxide solution. A ruby-red color results which soon turns yellow. See Legal's test for acetone, page 305.

✓ 3. **Salkowski's Test.**—To the yellow solution obtained in Weyl's test above add an excess of acetic acid and apply heat. A green color results and is in turn displaced by a blue color. A precipitate of Prussian blue may form.

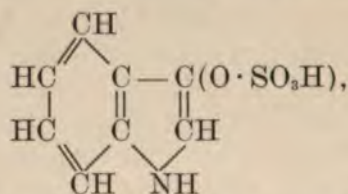
✓ 4. **Jaffe's Reaction.**—Place 5 c.c. of urine in a test-tube, add an aqueous solution of picric acid and render the mixture alkaline with potassium hydroxide solution. A red color is produced which turns yellow if the solution be acidified. Dextrose gives a similar red color but only upon the application of heat. This color reaction observed when creatinin in alkaline solution is treated with picric acid is the basic principle of Folin's colorimetric method for the quantitative determination of creatinin (see page 369).

ETHEREAL SULPHURIC ACIDS.

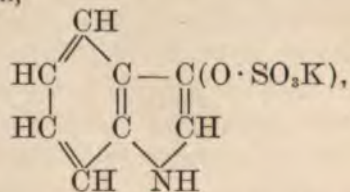
The most important of the ethereal sulphuric acids found in the urine are *phenol-sulphuric acid*, *p-cresol-sulphuric acid*, *indoxyl-sulphuric acid* and *skatoxyl-sulphuric acid*. Pyrocatechin-sulphuric acid also occurs in traces in human urine. The total output of ethereal sulphuric acid varies from 0.09 to 0.62 gram for 24 hours. In health the ratio of ethereal sulphuric acid to inorganic sulphuric acid is about 1 : 10. These ethereal sulphuric acids originate in part from the phenol, cresol, indol and skatol formed in the putrefaction of proteid material in the intestine. The phenol passes into the urine directly as the corresponding ethereal sulphuric acid whereas the indol and skatol undergo a preliminary oxidation to form *indoxyl* and *skatoxyl* respectively before their elimination.

It has generally been considered that each of the ethereal sulphuric acids was formed principally in the putrefaction of proteid material in the intestine and that therefore a determination of the total ethereal sulphuric acid content of the urine was an index of the extent to which these putrefactive proc-

esses were proceeding within the organism. Recently, however, Folin has conducted a series of experiments which seem to show that the ethereal sulphuric acid content of the urine does *not* afford an index of the extent of intestinal putrefaction, since these bodies arise only in part from putrefactive processes. He claims that the ethereal sulphuric acid excretion represents a form of sulphur metabolism which is more in evidence upon a diet containing a very small amount of proteid or upon a diet containing absolutely no proteid. The ethereal sulphuric acid content of the urine diminishes as the total sulphur content diminishes but the *percentage decrease* is much less. Therefore when considered from the standpoint of the total sulphuric acid content the ethereal sulphuric acid content is not diminished but *is increased*, although the total sulphuric acid content *is diminished*. Folin's experiments also seem to show that the indoxyl sulphuric acid (potassium indoxyl sulphate or indican) content of the urine does not originate to any degree from the metabolism of proteid material but that it arises in great part from intestinal putrefaction and that the excretion of indoxyl sulphuric acid may *alone* be taken as a rough index of the extent of putrefactive processes within the intestine. Indoxyl sulphuric acid,



therefore, which occurs in the urine as potassium indoxyl sulphate or indican,

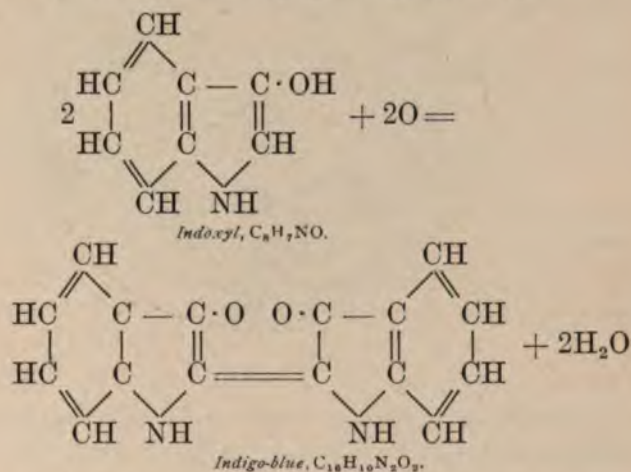


is clinically the most important of the ethereal sulphuric acids.

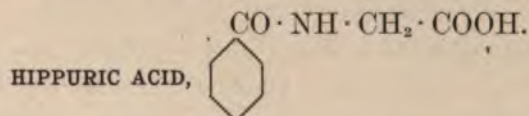
TESTS FOR INDICAN.

1. **Jaffe's Test.**—Nearly fill a test-tube with a mixture composed of equal volumes of concentrated HCl and the urine under examination. Add 2–3 c.c. of chloroform and a few drops of a calcium hypochlorite solution, place the thumb over the end of the test-tube and shake thoroughly. The chloroform is colored more or less, according to the amount of indican present. Ordinarily a blue color due to the formation of indigo-blue is produced; less frequently a red color due to indigo-red may be noted.

This is the reaction (see also pages 129 and 130):



2. **Obermayer's Test.**—Nearly fill a test-tube with a mixture composed of equal volumes of Obermayer's reagent¹ and the urine under examination. Add 2–3 c.c. of chloroform, place the thumb over the end of the test-tube and shake thoroughly. How does this compare with Jaffe's test?



¹ Obermayer's reagent is prepared by adding 2–4 grams of ferric chloride to a liter of concentrated HCl (sp. gr. 1.19).

This acid occurs normally in the urine of both the carnivora and herbivora but is more abundant in the urine of the latter. It is formed by a synthesis of benzoic acid and glycocholl which takes place in the kidneys. The average excretion of an adult man for 24 hours under normal conditions is about 0.7 gram. Hippuric acid crystallizes in needles or rhombic prisms (see

FIG. 92.



HIPPURIC ACID.

Fig. 92, above), the particular form depending upon the rapidity of crystallization. It is easily soluble in alcohol or hot water, and only slightly soluble in ether. The output of hippuric acid is increased in diabetes owing probably to the ingestion of much proteid and fruit. It is decreased in fevers and in certain kidney disorders where the synthetic activity of the renal cells is diminished.

EXPERIMENTS ON HIPPURIC ACID.

1. **Separation from the Urine.**—Render 500–1000 c.c. of urine of the horse or cow¹ alkaline with milk of lime, boil for

¹ If urine of the horse or cow is not available human urine may serve the purpose fully as well provided means are taken to increase its content of hippuric acid. This may be conveniently accomplished by ingesting 2

a few moments and filter while hot. Concentrate the filtrate, over a burner, to a small volume. Cool the solution, acidify it strongly with concentrated hydrochloric acid and stand it in a cool place for 24 hours. Filter off the crystals of hippuric acid which have formed and wash them with a little cold water. Remove the crystals from the paper, dissolve them in a very small amount of hot water and percolate the hot solution through thoroughly washed animal charcoal, being careful to wash out the last portion of the hippuric acid solution with hot water. Filter, concentrate the filtrate to a small volume and stand it aside for crystallization. Examine the crystals under the microscope and compare them with those in Fig. 92, page 256.

2. **Melting-Point.**—Determine the melting-point of the hippuric acid prepared in the above experiment (see p. 243).

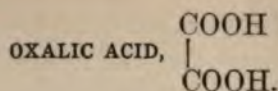
3. **Solubility.**—Test the solubility of hippuric acid in the ordinary solvents (page 4) and in alcohol, and ether.

4. **Formation of Nitro-Benzene.**—To a little hippuric acid in a small porcelain dish add 1–2 c.c. of concentrated HNO_3 and evaporate to dryness on a water-bath. Transfer the residue to a dry test-tube, apply heat and note the odor of the artificial oil of bitter almonds (nitro-benzene).

5. **Sublimation.**—Place a few crystals of hippuric acid in a dry test-tube and apply heat. The crystals are reduced to an oily fluid which solidifies in a crystalline mass upon cooling. When stronger heat is applied the liquid assumes a red color and finally yields a sublimate of benzoic acid and the odor of hydrocyanic acid.

6. **Formation of Ferric Salt.**—Render a small amount of a solution of hippuric acid neutral with dilute potassium hydroxide. Now add 1–3 drops of neutral ferric chloride solution and note the formation of the ferric salt of hippuric acid as a cream colored precipitate.

grams of ammonium benzoate at night. The fraction of urine passed in the morning will be found to have a high content of hippuric acid. The ammonium benzoate is in no way harmful.



Oxalic acid is a constituent of normal urine, about 0.02 gram being eliminated in 24 hours. It is present in the urine as calcium oxalate, which is kept in solution through the medium of the acid phosphates. The origin of the oxalic acid content of the urine is not well understood. It is eliminated, at least in part, unchanged when ingested, therefore since many of the common articles of diet, *e. g.*, asparagus, apples, cabbage, grapes, lettuce, spinach, tomatoes, etc., contain oxalic acid it seems probable that the ingested food supplies a portion of the oxalic acid found in the urine. There is also experimental evidence that part of the oxalic acid of the urine is formed within the organism in the course of proteid and fat metabolism. It has also been suggested that oxalic acid may arise from an incomplete combustion of carbohydrates, especially under certain abnormal conditions. Pathologically, oxalic acid is found to be increased in amount in diabetes mellitus, in organic diseases of the liver and in various other conditions which are accompanied by a derangement of the oxidation mechanism. An abnormal increase of oxalic acid is termed *oxaluria*. A considerable increase in the content of oxalic acid may be noted unaccompanied by any other apparent symptom. Calcium oxalate crystallizes in at least two distinct forms, *dumb-bells* and *octahedra* (Fig. 99, page 320).

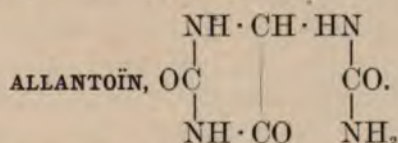
EXPERIMENTS.

1. **Preparation of Calcium Oxalate.**—Place 200–250 c.c. of urine in a beaker, add 10 drops of a saturated solution of oxalic acid and stand the beaker aside in a cool place for 24 hours. Examine the sediment under the microscope and compare the crystalline forms with those shown in Fig. 99, p. 320.

2. **Solubility.**—Test the solubility of calcium oxalate in the ordinary solvents (page 4) and in acetic and hydrochloric acids.

NEUTRAL SULPHUR COMPOUNDS.

Under this head may be classed such bodies as cystin (see p. 76), chondroitin-sulphuric acid, oxyproteic acid, alloxypoteic acid, uroferic acid, sulphocyanides and taurin derivatives. The sulphur content of the bodies just enumerated is generally termed loosely combined or neutral sulphur in order that it may not be confused with the acid sulphur which occurs in the inorganic sulphuric acid and ethereal sulphuric acid forms. Ordinarily the neutral sulphur content of normal human urine is 14-20 per cent of the total sulphur content.



Allantoïn has been found in the urine of suckling calves as well as in that of the dog and cat. It has also been detected in the urine of infants within the first eight days after birth, as well as in the urine of adults. It is more abundant in the urine

FIG. 93.



ALLANTOÏN, FROM CAT'S URINE.

a and *b*, Forms in which it crystallized from the urine; *c*, re-crystallized allantoïn. (Drawn from micro-photographs furnished by Prof. Lafayette B. Mendel of Yale University.)

of women during pregnancy. Allantoin is formed by the oxidation of uric acid and the output is increased by thymus or pancreas feeding. When pure it crystallizes in prisms (Fig. 93, p. 259) and when impure in granules and knobs. Pathologically, it has been found increased in diabetes insipidus and in hysteria with convulsions (Pouchet).

EXPERIMENTS.

1. **Separation from the Urine.**¹—*Meissner's Method.*—Precipitate the urine with baryta water. Neutralize the filtrate *carefully* with dilute sulphuric acid, filter immediately and evaporate the filtrate to incipient crystallization. Completely precipitate this *warm* fluid with 95 per cent alcohol (reserve the precipitate). Decant or filter and precipitate the solution by ether. Combine the ether and alcohol precipitates and extract with *cold* water or *hot* alcohol; allantoin remains undissolved. Bring the allantoin into solution in *hot* water and recrystallize.

Allantoin may be determined quantitatively by Loewi's method.²

2. **Preparation from Uric Acid.**—Dissolve 4 grams of uric acid in 100 c.c. of water rendered alkaline with potassium hydroxide. Cool and *carefully* add 3 grams of potassium permanganate. Filter, *immediately* acidulate the filtrate with acetic acid and allow it to stand in a cool place over night. Filter off the crystals and wash them with water. Save the wash water and filtrate, unite them and after concentrating to a small volume, stand away for crystallization. Now combine all the crystals and recrystallize them from hot water. Use these crystals in the experiments which follow.

3. **Microscopical Examination.**—Examine the crystals made in the last experiment and compare them with those shown in Fig. 93, page 259.

¹ The urine of the dog after thymus, pancreas or uric acid feeding may be employed.

² Archiv für Experimentelle Pathologie und Pharmakologie, 1900, xliv, p. 20.

4. **Solubility.**—Test the solubility of allantoin in the ordinary solvents (page 4).

5. **Reaction.**—Dissolve a crystal in water and test the reaction to litmus.

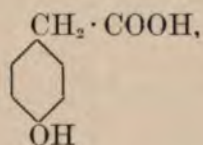
6. **Furfurol Test.**—Place a few crystals of allantoin on a test-tablet or in a porcelain dish and add 1–2 drops of a concentrated aqueous solution of furfurol and 1–2 drops of concentrated hydrochloric acid. Observe the formation of a yellow color which turns to a light purple if allowed to stand. This test is given by urea but not by uric acid.

7. **Murexid Test.**—Try this test according to the directions given on page 249. Note that allantoin fails to respond.

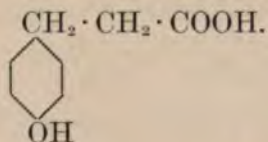
8. **Reduction of Fehling's Solution.**—Make this test in the usual way (see p. 286) except that the boiling must be prolonged and excessive. Ultimately the allantoin will reduce the solution. Compare with the result on uric acid, page 249.

AROMATIC OXYACIDS.

Two of the most important of the oxyacids are *paraoxyphenyl-acetic acid*,



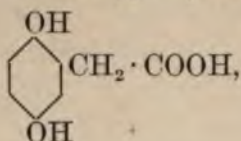
and *paraoxyphenyl-propionic acid*,



They are products of the putrefaction of proteid material and tyrosin is an intermediate stage in their formation. Both these acids for the most part pass unchanged into the urine where they occur normally in very small amount. The content may be increased in the same manner as the phenol con-

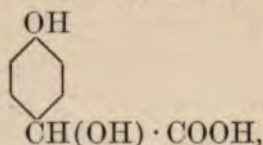
tent, in particular by acute phosphorus poisoning. A fraction of the total aromatic oxyacid content of the urine is in combination with sulphuric acid, but the greater part is present in the form of salts of sodium and potassium.

Homogentisic Acid or di-oxyphenyl-acetic acid.



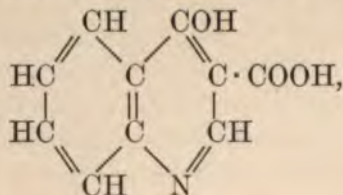
is another important oxyacid sometimes present in the urine. Under the name *glycosuric acid* it was first isolated from the urine by Prof. John Marshall of the University of Pennsylvania; subsequently Baumann isolated it and determined its chemical constitution. It occurs in cases of *alcaptonuria*. A urine containing this oxyacid turns greenish-brown from the surface downward when treated with a little sodium hydroxide or ammonia. If the solution be stirred the color very soon becomes dark brown or even black. Homogentisic acid reduces alkaline copper solutions but not alkaline bismuth solutions. Uroleucic acid is similar in its reactions to homogentisic acid.

Oxymandelic Acid or paraoxyphenyl-glycolic acid,



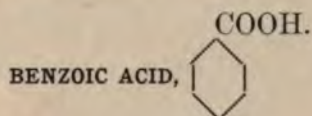
has been detected in the urine in cases of yellow atrophy of the liver.

Kynurenin Acid or γ -oxy- β -quinoline carbonic acid,



is present in the urine of the dog and has recently been detected by Swain in the urine of the coyote. To isolate it from the urine proceed as follows: Acidify the urine with hydrochloric acid in the proportion 1:25. From this acid fluid both the uric acid and the kynurenic acid separate in the course of 24-48 hours. Filter off the combined crystalline deposit of the two acids, dissolve the kynurenic acid in dilute ammonia (uric acid is insoluble) and reprecipitate it with hydrochloric acid.

Kynurenic acid may be quantitatively determined by Capaldi's method.¹



Benzoic acid has been detected in the urine of the rabbit and dog. It is also said to occur in human urine accompanying renal disorders. The benzoic acid probably originates from a fermentative decomposition of the hippuric acid of the urine.

EXPERIMENTS.

1. **Solubility.**—Test the solubility of benzoic acid in water, alcohol and ether.

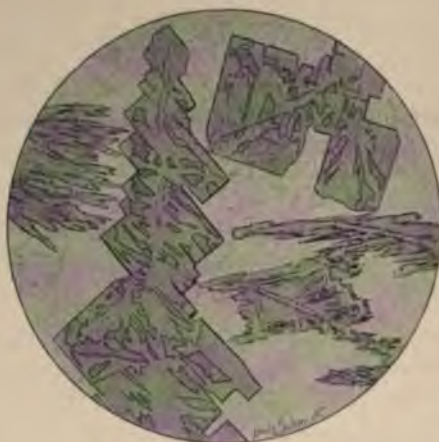
2. **Crystalline Form.**—Recrystallize some benzoic acid from hot water, examine the crystals under the microscope and compare them with those reproduced in Fig. 94, page 264.

3. **Sublimation.**—Place a little benzoic acid in a test-tube and heat over a flame. Note the odor which is evolved and observe that the acid sublimes in the form of needles.

4. Dissolve a little sodium benzoate in water and add a solution of neutral ferric chloride. Note the production of a brownish-yellow precipitate (Salicylic acid gives a reddish-violet color under the same conditions). Add ammonium hydroxide to some of the precipitate. It dissolves and ferric hydroxide is formed. Add a little hydrochloric acid to another portion of the original precipitate and stand the vessel away over night. What do you observe?

¹ Zeitschrift für physiologische Chemie, 1897, xxiii, p. 92.

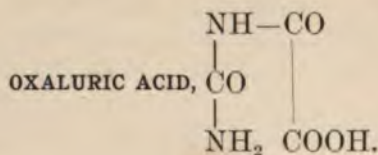
FIG. 94.



BENZOIC ACID.

NUCLEO-PROTEID.

The nubecula of normal urine has been shown by one investigator to consist of a mucoid containing 12.7 per cent of nitrogen and 2.3 per cent of sulphur. This body evidently originates in the urinary passages. It is probably slightly soluble in the urine. Some investigators believe that the body forming the nubecula of normal urine is nucleo-proteid and not a mucin or mucoid as stated above. A discussion of nucleo-proteid and related bodies occurring in the urine under pathological conditions will be found on page 296.



Oxaluric acid is not a constant constituent of normal human urine, and when found occurs only in traces as the ammonium salt. Upon boiling oxaluric acid it splits into oxalic acid and urea.

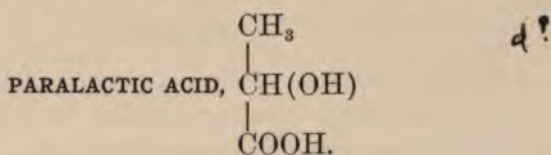
ENZYMES.

Various types of enzymes have been isolated from the urine. Pepsin, which probably originates in the stomach, and a diastatic enzyme have been more carefully studied than the other forms. The presence of trypsin and rennin in the urine is questioned.

VOLATILE FATTY ACIDS.

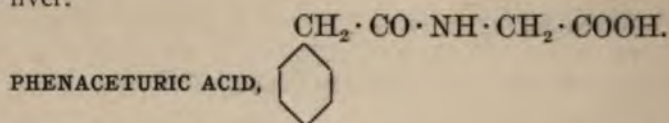
Acetic, butyric and formic acids have been found under normal conditions in the urine of man and of certain carnivora as well as in the urine of herbivora. Normally they arise principally from the fermentation of carbohydrates and the putrefaction of proteids. The acids containing the fewest carbon atoms (formic and acetic) are found to be present in larger percentage than those which contain a larger number of such atoms. The volatile fatty acids occur in normal urine in traces, the total output for twenty-four hours, according to different investigators, varying from 0.008 gram to 0.05 gram.

Pathologically, the excretion of volatile fatty acids is increased in diabetes, fevers, and in certain hepatic diseases in which the parenchyma of the liver is seriously affected. Under other pathological conditions the output may be diminished. These variations, however, in the excretion of the volatile fatty acids possess very little diagnostic value.



Paralactic acid is supposed to pass into the urine when the supply of oxygen in the organism is diminished through any cause, *e. g.*, after acute yellow atrophy of the liver, acute phosphorus poisoning or epileptic attacks. This acid has also been found in the urine of healthy persons following the physical exercise incident to prolonged marching. Paralactic

acid has been detected in the urine of birds after the removal of the liver.



Phenaceturic acid occurs principally in the urine of herbivorous animals but has frequently been detected in human urine. It is produced in the organism through the synthesis of glycoll and phenylacetic acid. It may be decomposed into its component parts by boiling with dilute mineral acids. The crystalline form of phenaceturic acid (small rhombic plates with rounded angles) resembles one form of uric acid crystal.

PHOSPHORIZED COMPOUNDS.

Phosphorus in organic combination has been found in the urine in such bodies as glycerophosphoric acid, which may arise from the decomposition of lecithin, and phosphocarnic acid. It is claimed that on the average about 2.5 per cent of the total phosphorus elimination is in organic combination.

PIGMENTS.

There are at least three pigments normally present in human urine. These pigments are *urochrome*, *urobilin* and *uroerythrin*.

A. UROCHROME.

This is the principal pigment of normal urine and imparts the characteristic yellow color to that fluid. It is apparently closely related to its associated pigment urobilin since the latter may be readily converted into urochrome through evaporation of its aqueous-ether solution. Urochrome may be obtained in the form of a brown, amorphous powder which is readily soluble in water and 95 per cent alcohol. It is less soluble in absolute alcohol, acetone, amyl alcohol and acetic ether and insoluble in benzene, chloroform and ether. Urochrome is said to be a nitrogenous body (4.2 per cent nitrogen), free from iron.

B. UROBILIN.

Urobilin, which was at one time considered to be the principal pigment of urine, in reality contributes little toward the pigmentation of this fluid. It is claimed that no urobilin is present in freshly voided normal urine but that its precursor, a chromogen called *urobilinogen*, is present and gives rise to urobilin upon decomposition through the influence of light. It is claimed by some investigators that there are various forms of urobilin, *e. g.*, normal, febrile, physiological and pathological. Urobilin is said to be very similar to, if not absolutely identical with, hydrobilirubin (see page 140).

Urobilin may be obtained as an amorphous powder which varies in color from brown to reddish-brown, red and reddish-yellow depending upon the way in which it is prepared. It is easily soluble in ethyl alcohol, amyl alcohol and chloroform, and slightly soluble in ether, acetic ether and in water. Its solutions show characteristic absorption-bands (see Absorption Spectra, Plate II). Under normal conditions urobilin is derived from the bile pigments in the intestine.

Urobilin is increased in most acute infectious diseases such as *erysipelas*, *malaria*, *pneumonia* and *scarlet fever*. It is also increased in *appendicitis*, *carcinoma of the liver*, *catarrhal icterus*, *pernicious anæmia* and in cases of poisoning by anti-febrin, antipyrin, pyridin, and potassium chlorate. In general it is usually increased when blood destruction is excessive and in many disturbances of the liver. It is markedly decreased in phosphorus poisoning.

EXPERIMENTS.

1. **Spectroscopic Examination.**—Acidify the urine with HCl and allow it to remain exposed to the air for a few moments. By this means if any urobilinogen is present it will be transformed into urobilin. The urine may now be examined by means of the spectroscope. If urobilin is present in the fluid the characteristic absorption-band lying between *b* and *F* will be observed (see Absorption Spectra, Plate II). It

may be found necessary to dilute the urine with water before a distinct absorption-band is observed. This test may be modified by acidifying 10 c.c. of urine with HCl and shaking it gently with 5 c.c. of amyl alcohol. The alcoholic extract when examined spectroscopically will show the characteristic urobilin absorption-band. (Note the spectroscopic examination in the next experiment.)

2. **Ammoniacal-Zinc Chloride Test.**—Render some of the urine ammoniacal by the addition of ammonium hydroxide, and after allowing it to stand a short time filter off the precipitate of phosphates and add a few drops of zinc chloride solution to the filtrate. Observe the production of a greenish fluorescence. Examine the fluid by means of the spectroscope and note the absorption-band which occupies much the same position as the absorption-band of urobilin in acid solution (see Absorption Spectra, Plate II).

3. **Gerhardt's Test.**—To 20 c.c. of urine add 3–5 c.c. of chloroform and shake well. Separate the chloroform extract and add to it a few drops of iodine solution (I in KI). Render the mixture alkaline with a dilute solution of potassium hydroxide and note the production of a yellow or yellowish-brown color. The solution ordinarily exhibits a greenish fluorescence.

4. **Wirsing's Test.**—To 20 c.c. of urine add 3–5 c.c. of chloroform and shake gently. Separate the chloroform extract and add to it a drop of an alcoholic solution of zinc chloride. Note the rose-red color and the greenish fluorescence. If the solution is turbid it may be rendered clear by the addition of a few c.c. of absolute alcohol.

5. **Ether-Absolute Alcohol Test.**—Mix urine and pure ether in equal volumes and shake gently in a separatory funnel. Separate the ether extract, evaporate it to dryness and dissolve the residue in 2–3 c.c. of absolute alcohol. Note the greenish fluorescence. Examine the solution spectroscopically and observe the characteristic absorption-band (see Absorption Spectra, Plate II).

6. **Ring Test.**—Acidify 25 c.c. of urine with 2-3 drops of concentrated HCl, add 5 c.c. of chloroform and shake the mixture. Separate the chloroform, place it in a test-tube and add carefully 3-5 c.c. of an alcoholic solution of zinc acetate. Observe the formation of a green ring at the zone of contact of the two fluids. If the tube is shaken a fluorescence may be observed.

C. UROERYTHRIN.

This pigment is frequently present in small amount in normal urine. The red color of urinary sediments is due in great part to the presence of uroerythrin. It is easily soluble in amyl alcohol, slightly soluble in acetic ether, absolute alcohol or chloroform, and nearly insoluble in water. Dilute solutions of uroerythrin are pink in color while concentrated solutions are orange-red or bright red: none of its solutions fluoresce. Uroerythrin is increased in amount after strenuous physical exercise, digestive disturbances, fevers, certain liver disorders and in various other pathological conditions.

PTOMAINES AND LEUCOMAINES.

These toxic substances are said to be present in small amount in normal urine. It is claimed that five different poisons may be detected in the urine, and it is further stated that each of these substances produces a specific and definite symptom when injected intravenously into a rabbit. The resulting symptoms are narcosis, salivation, mydriasis, paralysis and convulsions. The day urine is principally narcotic and is 2-4 times as toxic as the night urine which is chiefly productive of convulsions.

PURIN BASES.

The purin bases found in human urine are adenin, carnin, epiguanin, episarkin, guanin, xanthin, heteroxanthin, hypoxanthin, paraxanthin and 1-methylxanthin. The main bulk of the purin base content of the urine is made up of *paraxanthin*, *heteroxanthin* and *1-methylxanthin* which are derived for the

most part from the caffein, theobromin and theophyllin of the food. The total purin base content is made up of the products of two distinct forms of metabolism, *i. e.*, metabolism of ingested nucleins and purins and metabolism of tissue nuclein material. Purin bases resulting from the first form of metabolism are said to be of *exogenous* origin whereas those resulting from the second form of metabolism are said to be of *endogenous* origin. The daily output of purin bases by the urine is extremely small and varies greatly with the individual (16-60 milligrams). The output is increased after the ingestion of nuclein material as well as after the increased destruction of leucocytes. A well marked increase accompanies leukaemia. Edsall has very recently shown that the output of purin bases by the urine is increased as a result of X-ray treatment.

EXPERIMENT.

1. **Formation of the Silver Salts.**—Add an excess of magnesia mixture¹ to 25 c.c. of urine. Filter off the precipitate and add ammoniacal silver solution² to the filtrate. A precipitate composed of the silver salts of the various purin bases is produced.

2. Inorganic Physiological Constituents.

Ammonia.

Next to urea, ammonia is the most important of the nitrogenous end-products of proteid metabolism. Ordinarily about 4.6-5.6 per cent of the total nitrogen of the urine is eliminated as ammonia and on the average this would be about 0.7 gram per day. Under normal conditions the ammonia is present in the urine in the form of the *chloride*, *phosphate* or *sulphate*. This is due to the fact that combinations of this sort are not

¹ Magnesia mixture may be prepared as follows: Dissolve 175 grams of MgSO_4 and 350 grams of NH_4Cl in 1400 c.c. of distilled water. Add 700 grams of concentrated NH_4OH , mix very thoroughly and preserve the mixture in a glass-stoppered bottle.

² Ammoniacal silver solution may be prepared according to directions given on page 377.

oxidized in the organism to form urea, but are excreted as such. This explains the increase in the output of ammonia which follows the administration of the ammonium salts of the mineral acids or of the acids themselves. On the other hand when ammonium acetate and many other ammonium salts of certain organic acids are administered no increase in the output of ammonia occurs since the salt is oxidized and its nitrogen ultimately appears in the urine as urea.

The acids formed during the process of proteid destruction within the body have an influence upon the excretion of ammonia similar to that exerted by acids which have been administered. Therefore a pathological increase in the output of ammonia is observed in such diseases as are accompanied by an increased and imperfect proteid metabolism, and especially in diabetes, in which disease diacetic acid and β -oxybutyric acid are found in the urine in combination with the ammonia.

As the result of recent experiments Folin claims that a pronounced decrease in the extent of proteid metabolism, as measured by the total nitrogen in the urine, is frequently accompanied by a decreased elimination of ammonia. The ammonia elimination is therefore probably determined by other factors than the total proteid catabolism as such. Furthermore, he believes that a decided decrease in the total nitrogen excretion is always accompanied by a *relative increase* in the ammonia-nitrogen, provided the food is of a character yielding an alkaline ash.

The quantitative determination of ammonia must be made upon the fresh urine since upon standing the normal urine will undergo ammoniacal fermentation (see page 230).

Sulphates.

Sulphur in combination, is excreted in two forms in the urine; first, as *loosely combined, unoxidized* or *neutral sulphur* and second, as *oxidized* or *acid sulphur*. The *loosely combined* sulphur is excreted mainly as a constituent of such bodies as cystin, cystein, taurin, hydrogen sulphide, ethyl sulphide, sul-

phocyanides, sulphonic acids, oxyproteic acid, alloxypoteic acid and uroferic acid. The amount of loosely combined sulphur eliminated is in great measure independent of the extent of proteid decomposition or of the total sulphur excretion. In this characteristic it is somewhat similar to the excretion of creatinin. The *oxidized* sulphur is eliminated in the form of sulphuric acid, principally as salts of sodium, potassium, calcium and magnesium; a relatively small amount occurs in the form of *etheral* sulphuric acid, *i. e.*, sulphuric acid in combination with such *aromatic* bodies as phenol, indol, skatol, cresol, pyrocatechin and hydroquinone. Sulphuric acid in combination with Na, K, Ca or Mg is sometimes termed *inorganic* or *preformed sulphuric acid* whereas the etheral sulphuric acid is sometimes called *conjugate sulphuric acid*. The greater part of the sulphur is eliminated in the oxidized form but the absolute percentage of sulphur excreted as the preformed, etheral or loosely combined type depends upon the total quantity of sulphur present, *i. e.*, there is no definite ratio between the three forms of sulphur which will apply under all conditions. The preformed sulphuric acid may be precipitated directly from acidified urine with BaCl_2 , whereas the etheral sulphuric acid must undergo a preliminary boiling in the presence of a mineral acid before it can be so precipitated.

The sulphuric acid excreted by the urine arises principally from the oxidation of proteid material within the body; a relatively small amount is due to ingested sulphates. Under normal conditions about 2.5 grams of sulphuric acid is eliminated daily. Since the sulphuric acid content of the urine has, for the most part, a proteid origin and since one of the most important constituents of the proteid molecule is nitrogen, it would be reasonable to suppose that a fairly definite ratio might exist between the excretion of these two elements. However, when we appreciate that the percentage content of N and S present in different proteids is subject to rather wide variations, the fixing of a ratio which will express the exact relation existing between these two substances, as they appear in the

urine as end-products of proteid metabolism, is practically impossible. It has been suggested that the ratio 5:1 expresses this relation in a general way.

Pathologically, the excretion of sulphuric acid by the urine is increased in acute fevers and in all other diseases marked by a stimulated metabolism, whereas a decrease in the sulphuric acid excretion is observed in those diseases which are accompanied by a loss of appetite and a diminished metabolic activity.

EXPERIMENTS.

1. **Detection of Inorganic Sulphuric Acid.**—Place about 10 c.c. of urine in a test-tube, acidify with acetic acid and add some barium chloride solution. A white precipitate of barium sulphate forms.

2. **Detection of Ethereal Sulphuric Acid.**—Filter off the barium sulphate precipitate formed in the above experiment, add 1 c.c. of hydrochloric acid and a little barium chloride solution to the filtrate and heat the mixture to boiling for 1–2 minutes. Note the appearance of a turbidity due to the presence of sulphuric acid which has been separated from the ethereal sulphates and has combined with the barium of the BaCl_2 to form BaSO_4 .

3. **Detection of Loosely Combined or Neutral Sulphur.**—Place about 10 c.c. of urine in a test-tube, introduce a small piece of zinc, add sufficient hydrochloric acid to cause a gentle evolution of hydrogen and over the mouth of the tube place a filter paper saturated with plumbic acetate solution. In a short time the portion of the paper in contact with the vapors within the test-tube becomes blackened due to the formation of lead sulphide. The nascent hydrogen has reacted with the loosely combined or neutral sulphur to form hydrogen sulphide and this gas coming in contact with the plumbic acetate paper has caused the production of the black lead sulphide. Sulphur in the form of inorganic or ethereal sulphuric acid does not respond to this test.

4. **Calcium Sulphate Crystals.**—Place 10 c.c. of urine in a

test-tube, add 10 drops of calcium chloride solution and allow the tube to stand until crystals form. Examine the calcium sulphate crystals under the microscope and compare them with those shown in Fig. 95, p. 274.

FIG. 95.



CALCIUM SULPHATE. (Hensel and Weil.)

Chlorides.

Next to urea, the chlorides constitute the chief *solid* constituent of the urine. The principal chlorides found in the urine are those of sodium, potassium, ammonium and magnesium, with sodium chloride predominating. The excretion of chlorides is dependent, in

great part, upon the nature of the diet, but on the average the daily output is about 10–15 grams, expressed as sodium chloride. Copious water-drinking increases the output of chlorides considerably. Because of their solubility, chlorides are never found in the urinary sediment.

Since the amount of chlorides excreted in the urine is due primarily to the chloride content of the food ingested, it follows that a decrease in the amount of ingested chloride will likewise cause a decrease in the chloride content of the urine. In cases of actual fasting the chloride content of the urine may be decreased to a *slight trace* which is derived from the body fluids and tissues. Under these conditions, however, an examination of the blood of the fasting subject will show the percentage of chlorides in this fluid to be approximately normal. This forms a very striking example of the care nature takes to maintain the normal composition of the blood. There is a limit to the power of the body to maintain this equilibrium, however, and if the fasting organism be subjected to the influence of diuretics for a time, a point is reached where the composition of the blood can no longer be maintained and a gradual decrease in its chloride content occurs which finally results in

death. Death is supposed to result not so much because of a lack of chlorine as from a *deficiency of sodium*. This is shown from the fact that potassium chloride, for instance, cannot replace the sodium chloride of the blood when the latter is decreased in the manner above stated. When this substitution is attempted the potassium salt is excreted at once in the urine, and death follows as above indicated.

Pathologically, the excretion of chlorides may be decreased in some fevers, chronic nephritis, croupous pneumonia, diarrhoea, certain stomach disorders and in acute articular rheumatism.

EXPERIMENT.

Detection of Chlorides in Urine.—Place about 5 c.c. of urine in a test-tube, render it acid with nitric acid and add a few drops of a solution of argentic nitrate. A white precipitate, due to the formation of argentic chloride, is produced. This precipitate is soluble in ammonium hydroxide.

Phosphates.

Phosphoric acid exists in the urine in two general forms: First, that in combination with the alkali metals, sodium and potassium, and the radical ammonium; second, that in combination with the alkaline earths, calcium and magnesium. Phosphates formed through a union of phosphoric acid with the alkali metals are termed *alkaline phosphates*, or phosphates of the alkali metals, whereas phosphates formed through a union of phosphoric acid with the alkaline earths are termed *earthy phosphates*, or phosphates of the alkaline earths.

Three series of salts are formed by phosphoric acid: *Normal*, M_3PO_4 ,¹ *mono-hydrogen*, M_2HPO_4 , and *di-hydrogen*, MH_2PO_4 . The di-hydrogen salts are acid in reaction and it was generally believed that about 60 per cent of the total phosphate content of the urine was in the form of this type of salt, and that the acidity of the urine was due in great part to the presence of *sodium di-hydrogen phosphate*. Re-

¹ M may be occupied by any of the alkali metals or alkaline earths.

cently, however, it has been quite clearly shown that the normal acidity of the urine is not due to the presence of this salt but is due, at least in part, to the presence of various acidic radicals. In this connection Folin believes that the phosphates in clear acid urine are *all* of the *mono-hydrogen* type, and that the acidity of the urines of this character is generally greater than the combined acidity of all the phosphates present; the excess in the acidity above that due to phosphates he believes to be due to *free organic acids*. In bones the phosphates occur principally in the form of the *normal* salts of calcium and magnesium. The mono-hydrogen salts as a class are alkaline in reaction to litmus, and it is to the presence of *di-sodium hydrogen phosphate*, Na_2HPO_4 , that the greater part of the alkalinity of the saliva is due.

The excretion of phosphoric acid is extremely variable but on the average the total output for 24 hours is about 2.5 grams, expressed as P_2O_5 . Ordinarily the total output is distributed between alkaline phosphates and earthy phosphates approximately in the ratio 2:1. The greater part of this phosphoric acid arises from the ingested food, either from the preformed phosphates or more especially from the phosphorus in organic combination such as we find it in *phospho-proteids*, *nucleo-proteids*, *nucleins* and *lecithins*; the phosphorus-containing tissues of the body also contribute to the total output of this element. Alkaline phosphates ingested with the food have a tendency to increase the phosphoric acid content of the urine to a greater extent than the earthy phosphates so ingested. This is due, in a measure, to the fact that a portion of the earthy phosphates, under certain conditions, may be precipitated in the intestine and excreted in the feces; this is especially to be noted in the case of herbivorous animals. Since the extent to which the phosphates are absorbed in the intestine depends upon the form in which they are present in the food, under ordinary conditions, there can be no absolute relationship between the urinary output of nitrogen and phosphorus. If

the diet is constant, however, from day to day, thus allowing of the preparation of both a nitrogen and a phosphorus balance,¹ a definite ratio may be established. In experiments upon dogs, which were fed an exclusive meat diet, the ratio of nitrogen to phosphorus, in the urine and feces, was found to be 8.1:1.

Pathologically the excretion of phosphoric acid is increased in such diseases of the bones as diffuse periostosis, osteomalacia and rickets; according to some investigators, in the early stages of pulmonary tuberculosis; in acute yellow atrophy of the liver; in diseases which are accompanied by an extensive decomposition of nervous tissue and after sleep induced by potassium bromide or chloral hydrate (Mendel). It is also increased after copious water-drinking. A decrease in the excretion of phosphates is at times noted in febrile affections, such as the acute infectious diseases; in pregnancy, in the period during which the foetal bones are forming, and in diseases of the kidneys, because of non-elimination.

EXPERIMENTS.

1. **Formation of "Triple Phosphate."**—Place some urine in a beaker, render it alkaline with ammonium hydroxide, add a small amount of magnesium sulphate solution and allow the beaker to stand in a cool place over night. Crystals of *ammonium magnesium phosphate*, "triple phosphate," form under these conditions. Examine the crystalline sediment under the microscope and compare the forms of the crystals with those shown in Fig. 96, page 278.

2. **"Triple Phosphate" Crystals in Ammoniacal Fermentation.**—Stand some urine aside in a beaker for several days. Ammoniacal fermentation will develop and "triple phosphate" crystals will form. Examine the sediment under

¹ In metabolism experiments, a statement showing the relation existing between the nitrogen content of the food on the one hand and that of the urine and feces on the other, for a definite period, is termed a *nitrogen balance* or a "balance of the income and outgo of nitrogen."

FIG. 96.



"TRIPLE PHOSPHATE." (Ogden.)

the microscope and compare the crystals with those shown in Fig. 96, above.

3. **Detection of Earthy Phosphates.**—Place 10 c.c. of urine in a test-tube and render it alkaline with ammonium hydroxide. Warm the mixture and note the separation of a precipitate of *earthy phosphates*.

4. **Detection of Alkaline Phosphates.**—Filter off the earthy phosphates as formed in the last experiment, and add a small amount of magnesia mixture (see page 270) to the filtrate. Now warm the mixture and observe the formation of a white precipitate due to the presence of *alkaline phosphates*. Note the difference in the size of the precipitates of the two forms of phosphates from this same volume of urine. Which form of phosphates were present in the larger amount, *earthy* or *alkaline*?

5. **Influence upon Fehling's Solution.**—Place 2 c.c. of Fehling's solution in a test-tube, dilute it with 4 volumes of water and heat to boiling. Add a solution of sodium dihydrogen phosphate, NaH_2PO_4 , a small amount at a time, and heat after each addition. What do you observe? What does this observation force you to conclude regarding the interference of phosphates in the testing of *diabetic urine* by means of Fehling's test?

Sodium and Potassium.

The elements sodium and potassium are always present in the urine. Usually they are combined with such acidic radicals as Cl , CO_3 , SO_4 and PO_4 . The amount of potassium, expressed as K_2O , excreted in 24 hours by an adult, subsisting upon a mixed diet, is on the average 2-3 grams, whereas the amount of sodium, expressed as Na_2O , under the same conditions, is ordinarily 4-6 grams. The ratio of K to Na is generally about 3:5. The absolute quantity of these elements excreted, depends, of course, in large measure, upon the nature of the diet. Because of the non-ingestion of NaCl and the accompanying destruction of potassium-containing body tissues, the urine during fasting contains more potassium salts than sodium salts.

Pathologically the output of potassium, in its relation to sodium, may be increased during fever; following the crisis, however, the output of this element may be decreased. It may also be increased in conditions associated with *acid intoxication*.

Calcium and Magnesium.

The greater part of the calcium and magnesium excreted in the urine is in the form of phosphates. The daily output, which depends principally upon the nature of the diet, aggregates on the average about 1 gram and is made up of the phosphates of calcium and magnesium in the proportion 1:2. The percentage of calcium salts present in the urine at any one time forms no dependable index as to the absorption of this class of salts, since they are again excreted into the intestine after absorption. It is therefore impossible to draw any satisfactory conclusions regarding the excretion of the alkaline earths unless we obtain accurate analytical data from both the feces and the urine.

Very little is known positively regarding the actual course of the excretion of the alkaline earths under pathological conditions except that an excess of calcium is found in *acid intoxication* and some diseases of the bones.

Carbonates.

Carbonates generally occur in small amount in the urine of man and carnivora under normal conditions, whereas much larger quantities are ordinarily present in the urine of herbivora. The alkaline reaction of the urine of herbivora is dependable in great measure upon the presence of carbonates. In general a urine containing carbonates in appreciable amount is turbid when passed or becomes so shortly after. These bodies ordinarily occur as alkali or alkaline earth compounds and the turbid character of urine containing them is usually due principally to the latter class of substances. The carbonates of the alkaline earths are often found in amorphous urinary sediments.

Iron.

Iron is present in small amount in normal urine. It probably occurs partly in inorganic and partly in organic combination. The iron contained in urinary pigments or chromogens is in organic combination. According to different investigators the iron content of normal urine varies from 0.012 gram to 0.15 gram per day.

EXPERIMENT.

Detection of Iron in Urine.—Evaporate a convenient volume (10–15 c.c.) of urine to dryness. Incinerate and dissolve the residue in a few drops of iron-free hydrochloric acid and dilute the acid solution with 5 c.c. of water. Divide the acid solution into two parts and make the following tests: (a) To the first part add a solution of ammonium sulphocyanide; a red color indicates the presence of iron. (b) To the second part of the solution add a little potassium ferrocyanide solution; a precipitate of Prussian blue forms upon standing.

Fluorides, Nitrates, Silicates and Hydrogen Peroxide.

These substances are all found in traces in human urine under normal conditions. Nitrates are undoubtedly intro-

duced into the organism in the water and ingested food. The average excretion of nitrates is about 0.5 gram per day, the output being the largest upon a vegetable diet and smallest upon a meat diet. Nitrites are found only in urine which is undergoing decomposition and are formed from the nitrates in the course of ammoniacal fermentation. Hydrogen peroxide has been detected in the urine, but its presence is believed to possess no pathological importance.

CHAPTER XVIII.

URINE: PATHOLOGICAL CONSTITUENTS.¹

Dextrose.											
Proteids	<table><tr><td rowspan="7">{</td><td>Serum albumin.</td></tr><tr><td>Serum globulin.</td></tr><tr><td>Proteoses { Deutero-proteose.</td></tr><tr><td>Hetero-proteose.</td></tr><tr><td>" Bence-Jones' proteid."</td></tr><tr><td>Peptone.</td></tr><tr><td>Nucleo-proteid.</td></tr><tr><td>Fibrin.</td></tr><tr><td>Hæmoglobin.</td></tr></table>	{	Serum albumin.	Serum globulin.	Proteoses { Deutero-proteose.	Hetero-proteose.	" Bence-Jones' proteid."	Peptone.	Nucleo-proteid.	Fibrin.	Hæmoglobin.
{	Serum albumin.										
	Serum globulin.										
	Proteoses { Deutero-proteose.										
	Hetero-proteose.										
	" Bence-Jones' proteid."										
	Peptone.										
	Nucleo-proteid.										
Fibrin.											
Hæmoglobin.											
Blood	<table><tr><td rowspan="2">{</td><td>Form elements.</td></tr><tr><td>Pigment.</td></tr></table>	{	Form elements.	Pigment.							
{	Form elements.										
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Bile	<table><tr><td rowspan="2">{</td><td>Pigments.</td></tr><tr><td>Acids.</td></tr></table>	{	Pigments.	Acids.							
{	Pigments.										
	Acids.										
Acetone.											
Diacetic acid.											
β -Oxybutyric acid.											
Conjugate glycuronates.											
Pentoses.											
Fat.											
Hæmatoporphyrin.											
Lactose.											
Lævulose.											
Inosit.											
Laiose.											
Melanin.											
Urorosein.											
Unknown substances.											

DEXTROSE.

Traces of this sugar occur in normal urine, but the amount is not sufficient to be readily detected by the ordinary simple

¹ See note at the bottom of page 237.

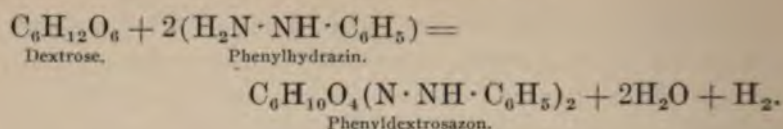
qualitative tests. There are two distinct types of *pathological glycosuria*, *i. e.*, transitory glycosuria and persistent glycosuria. The transitory type may follow the ingestion of an excess of sugar, causing the *assimilation limit* to be exceeded, or it may accompany any one of several disorders which cause an impairment of the power of assimilating sugar. In the persistent type large amounts of sugar are excreted daily in the urine for long periods of time. Under such circumstances a condition known as diabetes mellitus exists. Ordinarily, diabetic urine which contains a high percentage of sugar possesses a faint yellow color, a high specific gravity and a volume which is above normal.

EXPERIMENTS.

I. Phenylhydrazin Reaction.—Test the urine according to one of the following methods: (a) To a small amount of phenylhydrazin mixture, furnished by the instructor,¹ add 5 c.c. of the urine, shake well and heat on a boiling water-bath for one-half to three-quarters of an hour. Allow the tube to cool *slowly* and examine the crystals microscopically (Plate III., opposite page 5). If the solution has become too concentrated in the boiling process it will be light-red in color and no crystals will separate until it is diluted with water.

Yellow crystalline bodies called *osazons* are formed from certain sugars under these conditions, each individual sugar giving rise to an osazon of a definite crystalline form which is typical for that sugar. Each osazon has a definite melting-point, and as a further and more accurate means of identification it may be recrystallized and identified by the determination of its melting-point and nitrogen content. The reaction taking place in the formation of *phenyldextrosazon* is as follows:

¹This mixture is prepared by combining one part of phenylhydrazin-hydrochloride and two parts of sodium acetate, *by weight*. These are thoroughly mixed in a mortar.



(b) Place 5 c.c. of the urine in a test-tube, add 1 c.c. of phenylhydrazin-acetate solution furnished by the instructor,¹ and heat on a boiling water-bath for one-half to three-quarters of an hour. Allow the liquid to cool *slowly* and examine the crystals microscopically (Plate III., opposite p. 5).

The phenylhydrazin test has been so modified by Cipollina as to be of use as a *rapid clinical test*. The directions for this test are given in the next experiment.

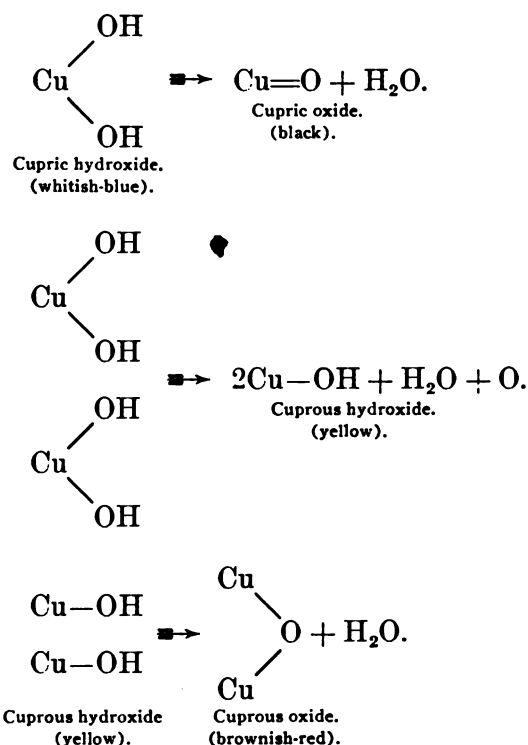
2. **Cipollina's Test.**—Thoroughly mix 4 c.c. of urine, 5 drops of phenylhydrazin (the base) and one-half c.c. of glacial acetic acid in a test-tube. Heat the mixture for about one minute over a low flame, shaking the tube continually to prevent loss of fluid by bumping. Add 4–5 drops of potassium hydroxide or sodium hydroxide (sp. gr. 1.16), being certain that the fluid in the test-tube remains acid; heat the mixture again for a moment and then cool the contents of the tube. Ordinarily the crystals form at once, especially if the urine possesses a low specific gravity. If they do not appear immediately allow the tube to stand at least 20 minutes before deciding upon the absence of sugar.

Examine the crystals under the microscope and compare them with those shown in Plate III., opposite page 5.

3. **Reduction Tests.**—To their aldehyde or ketone structure many sugars owe the property of readily reducing the alkaline solutions of the oxides of metals like copper, bismuth and mercury; they also possess the property of reducing ammoniacal silver solutions with the separation of metallic silver. Upon this property of reduction the most widely used tests for sugars are based. When whitish-blue cupric hydrox-

¹ This solution is prepared by mixing one part *by volume*, in each case, of glacial acetic acid, one part of water and two parts of phenylhydrazin (the base).

ide in suspension in an alkaline liquid is heated it is converted into insoluble black cupric oxide, but if a reducing agent like certain sugars be present the cupric hydroxide is reduced to insoluble yellow cuprous hydroxide, which in turn on further heating may be converted into brownish-red or red cuprous oxide. These changes are indicated as follows:



The chemical equations here discussed are exemplified in Trommer's and Fehling's tests.

(a) **Trommer's Test.**—To 5 c.c. of urine in a test-tube add one-half its volume of KOH or NaOH. Mix thoroughly and add, drop by drop, agitating after the addition of each drop, a *very dilute* solution of cupric sulphate. Continue the addition until there is a slight permanent precipitate of

cupric hydroxide and in consequence the solution is slightly turbid. Heat, and the cupric hydroxide is reduced to yellow cuprous hydroxide or to brownish-red cuprous oxide. If the solution of cupric sulphate used is too strong, a small brownish-red precipitate produced in the presence of a low percentage of dextrose may be entirely masked. On the other hand, if too little cupric sulphate is used a light-colored precipitate formed by uric acid and purin bases may obscure the brownish-red precipitate of cuprous oxide. The action of KOH or NaOH in the presence of an excess of sugar and insufficient copper will produce a brownish color. Phosphates of the alkaline earths may also be precipitated in the alkaline solution and be mistaken for cuprous hydroxide. Trommer's test is not very satisfactory.

(b) **Fehling's Test.**—To about 1 c.c. of Fehling's solution¹ in a test-tube add about 4 c.c. of water, and boil. This is done to determine whether the solution will of itself cause the formation of a precipitate of brownish-red cuprous oxide. If such a precipitate forms, the Fehling's solution must not be used. Add urine to the warm Fehling's solution, *a few drops* at a time, and heat the mixture after each addition. The production of yellow cuprous hydroxide or brownish-red cuprous oxide indicates that reduction has taken place. The yellow precipitate is more likely to occur if the urine is added rapidly and in large amount, whereas with a less rapid addition of smaller amounts of urine the brownish-red precipitate is generally formed.

This is a much more satisfactory test than Trommer's, but

¹Fehling's solution is composed of two definite solutions—a cupric sulphate solution and an alkaline tartrate solution, which may be prepared as follows:

Cupric sulphate solution = 34.64 grams of cupric sulphate dissolved in water and made up to 500 c.c.

Alkaline tartrate solution = 125 grams of potassium hydroxide and 173 grams of Rochelle salt dissolved in water and made up to 500 c.c.

These solutions should be preserved separately in rubber-stoppered bottles and mixed in equal volumes when needed for use. This is done to prevent deterioration.

even this test is not entirely reliable when used to detect sugar in the urine. Such bodies as *conjugate glycuronates*, *uric acid*, *nucleo-proteid* and *homogentisic acid*, when present in sufficient amount, may produce a result similar to that produced by sugar. Phosphates of the alkaline earths may be precipitated by the alkali of the Fehling's solution and in appearance may be mistaken for the cuprous hydroxide. Cupric hydroxide may also be reduced to cuprous oxide and this in turn be dissolved by *creatinin*, a normal urinary constituent. This will give the urine under examination a greenish tinge and may obscure the sugar reaction even when a considerable amount of sugar is present.

(c) **Allen's Modification of Fehling's Test.**—The following procedure is recommended: "From 7 to 8 c.c. of the sample of urine to be tested is heated to boiling in a test-tube, and, without separating any precipitate of albumin which may be produced, 5 c.c. of the solution of cupric sulphate used for preparing Fehling's solution is added. This produces a precipitate containing uric acid, xanthin, hypoxanthin, phosphates, etc. To render the precipitation complete, however, it is desirable to add to the liquid, *when partially cooled*, from 1 to 2 c.c. of a saturated solution of sodium acetate having a feebly acid reaction to litmus.¹ The liquid is filtered and to the filtrate, which will have a bluish-green color, 5 c.c. of the alkaline tartrate mixture used for preparing Fehling's solution is added, and the liquid boiled for 15–20 seconds. In the presence of more than 0.25 per cent of sugar, separation of cuprous oxide occurs before the boiling-point is reached; but with smaller quantities precipitation takes place during the cooling of the solution, which becomes green-

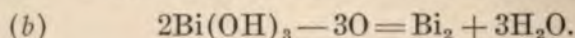
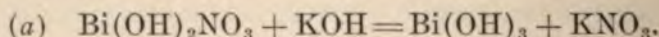
¹ Sufficient acetic acid should be added to the sodium acetate solution to render it feebly acid to litmus. A saturated solution of sodium acetate keeps well, but weaker solutions are apt to become mouldy, and then possess the power of reducing Fehling's solution. Hence it is essential in all cases of importance to make a blank test by mixing equal measures of cupric sulphate solution, alkaline tartrate solution and water, adding a little sodium acetate solution, and heating the mixture to boiling.

ish, opaque, and suddenly deposits cuprous oxide as a fine brownish-red precipitate."

(d) **Boettger's Test.**—To 5 c.c. of urine in a test-tube add 1 c.c. of KOH or NaOH and a very small amount of bismuth subnitrate, and boil. The solution will gradually darken and finally assume a black color due to reduced bismuth. If the test is made with urine containing albumin this must be removed, by boiling and filtering, before applying the test, since with albumin a similar change of color is produced (bismuth sulphide).

(e) **Nylander's Test (Almén's Test).**—To 5 c.c. of urine in a test-tube add one-tenth its volume of Nylander's reagent¹ and boil two or three minutes. The mixture will darken if reducing sugar is present and upon standing for a few moments a black color will appear. This color is due to the precipitation of bismuth. If the test is made on urine containing albumin this must be removed, by boiling and filtering, before applying the test. It is claimed by Bechold that Nylander's and Boettger's tests give a negative reaction with solutions containing sugar when mercuric chloride or chloroform is present, a claim which has very recently been contradicted by Zeidlitz.

A positive Nylander or Boettger test is probably due to the following reactions:



4. **Fermentation Test.**—Rub up in a mortar about 15 c.c. of the urine with a small piece of compressed yeast. Transfer the mixture to a saccharometer (Fig. 2, p. 10) and stand it aside in a warm place for about 12 hours. If dextrose is present, alcoholic fermentation will occur and carbon dioxide will collect as a gas in the upper portion of the tube. On the

¹ Nylander's reagent is prepared by digesting 2 grams of bismuth subnitrate and 4 grams of Rochelle salt in 100 c.c. of a 10 per cent potassium hydroxide solution. The reagent is then cooled and filtered.

completion of fermentation introduce, by means of a bent pipette, a little KOH solution into the graduated portion, place the thumb tightly over the opening in the apparatus and invert the saccharometer. Explain the result.

5. **Barfoed's Test.**—To 2–3 c.c. of Barfoed's solution¹ in a test-tube add a few drops of urine and boil. Allow the tube to stand a few minutes and examine. In the presence of dextrose a red precipitate forms. What is it?

6. **Polariscopic Examination.**—For directions as to the use of the polariscope see page 11.

PROTEIDS.

Normal urine contains a trace of proteid material but the amount present is so slight as to escape detection by any of the simple tests in general use for the detection of proteid urinary constituents. The following are the more important forms of proteid material which have been detected in the urine under pathological conditions:

- (1) Serum albumin.
- (2) Serum globulin.
- (3) Proteoses { Deutero-proteose.
Hetero-proteose.
"Bence-Jones' proteid."
- (4) Peptone.
- (5) Nucleo-proteid.
- (6) Fibrin.
- (7) Hæmoglobin.

ALBUMIN.

Albuminuria is a condition in which serum albumin or serum globulin appears in the urine. There are two distinct forms of albuminuria, *i. e.*, *renal* albuminuria and *accidental* albuminuria. Sometimes the terms "true" albuminuria and

¹ Barfoed's solution is prepared as follows: Dissolve 4 grams of cupric acetate in 100 c.c. of water and acidify with acetic acid.

"false" albuminuria are substituted for those just given. In the renal type the albumin is excreted by the kidneys. This is the more serious form of the malady and at the same time is more frequently encountered than the accidental type. Among the causes of renal albuminuria are altered blood pressure in the kidneys, altered kidney structure, or changes in the composition of the blood entering the kidneys, thus allowing the albumin to diffuse more readily. In the accidental form of albuminuria the albumin is *not* excreted by the kidneys as is the case in the renal form of the disorder, but arises from the blood, lymph or some albumin-containing exudate coming into contact with the urine at some point below the kidneys.

EXPERIMENTS.

1. **Heller's Ring Test.**—Place 5 c.c. of concentrated HNO_3 in a test-tube, incline the tube, and, by means of a pipette allow the urine to flow *slowly* down the side.¹ The liquids should stratify with the formation of a *white* zone of precipitated albumin at the point of juncture. If the albumin is present in very small amount the white zone may not form until the tube has been allowed to stand for several minutes. If the urine is quite concentrated a white zone, due to uric acid or urates, will form upon treatment with nitric acid as indicated. This ring may be easily differentiated from the albumin ring by repeating the test after diluting the urine with 3 or 4 volumes of water, whereupon, the ring, if due to uric acid or urates, will not appear. It is ordinarily possible to differentiate between the albumin ring and the uric acid ring without diluting the urine, since the ring, when due to uric acid, has ordinarily a less sharply defined upper border, is generally broader than the albumin ring and frequently is situated in the urine *above* the point of contact with the nitric acid. Concentrated urines also occasionally exhibit the formation, at the point of contact, of a *crystalline*

¹ An apparatus called the *albumoscope* has been devised for use in this test and has met with considerable favor.

ring with very sharply defined borders. This is urea nitrate and is easily distinguished from the "fluffy" ring of albumin. If there is any difficulty in differentiation a simple dilution of the urine with water, as above described, will remove the difficulty. Various colored zones, due either to the presence of indican, bile pigments or to the oxidation of other organic urinary constituents, may form in this test under certain conditions. These colored rings should never be confounded with the *white* ring which alone denotes the presence of albumin.

After the administration of certain drugs a white precipitate of *resin acids* may form at the point of contact of the two fluids and may cause the observer to draw wrong conclusions. This ring, if composed of resin acids, will dissolve in alcohol, whereas the albumin ring will not dissolve.

2. **Roberts' Ring Test.**—Place 5 c.c. of Roberts' reagent¹ in a test-tube, incline the tube, and, by means of a pipette, allow the urine to flow *slowly* down the side. The liquids should stratify with the formation of a *white* zone of precipitated albumin at the point of juncture. This test is a modification of Heller's ring test and is rather more satisfactory than that test, since the colored rings never form and the consequent confusion is avoided.

3. **Spiegler's Ring Test.**—Place 5 c.c. of Spiegler's reagent² in a test-tube, incline the tube, and, by means of a pipette, allow 5 c.c. of urine, acidified with acetic acid, to flow *slowly* down the side. A white zone will form at the point of contact. This is an exceedingly delicate test, in fact, too delicate for ordinary clinical purposes, since it serves to detect albumin when present in the merest trace (1:250,000) and hence most normal urines will give a positive reaction for albumin when this test is applied.

¹ Roberts' reagent is composed of 1 volume of concentrated HNO_3 and 5 volumes of a saturated solution of MgSO_4 .

² Spiegler's reagent has the following composition:

Tartaric acid.....	20 grams.
Mercuric chloride.....	40 grams.
Glycerin	100 grams.
Distilled water.....	1000 grams.

Some investigators claim that the delicacy of this test depends upon the presence of sodium chloride in the urine, the test losing accuracy if the sodium chloride content be low.

4. **Jolles' Reaction.**—Shake 5 c.c. of urine with 1 c.c. of 30 per cent acetic acid and 4 c.c. of Jolles' reagent¹ in a test-tube. A white precipitate indicates the presence of albumin.

Care should be taken to use the correct amount of acetic acid, since the use of too small an amount may result in the formation of mercury combinations which may cause confusion. In the presence of iodine, mercuric iodide will form but may readily be differentiated from albumin through the fact that it is *soluble* in alcohol.

5. **Coagulation or Boiling Test.**—(a) Heat 5 c.c. of urine to boiling in a test-tube. A precipitate forming at this point is due either to albumin or to phosphates. Acidify the urine slightly by the addition of 3–5 drops of very dilute acetic acid, adding the acid drop by drop to the *hot* solution. If the precipitate is due to phosphates it will disappear under these conditions, whereas if it is due to albumin it will not only fail to disappear but will become more flocculent in character, since the reaction of a fluid must be acid to secure the complete precipitation of the albumin by this coagulation process. Too much acid should be avoided since it will cause the albumin to go into solution. Certain *resin acids* may be precipitated by the acid, but the precipitate due to this cause may be easily differentiated from the albumin precipitate by reason of its solubility in alcohol.

(b) A modification of this test in quite general use is as follows: Fill a test-tube two-thirds full of urine and gently heat the *upper half* of the fluid to boiling, being careful that this fluid does not mix with the lower half. A turbidity indicates albumin or phosphates. Acidify the urine slightly by

¹ Jolles' reagent has the following composition:

Succinic acid.....	40 grams.
Mercuric chloride.....	20 grams.
Sodium chloride.....	20 grams.
Distilled water.....	1000 grams.

the addition of 3-5 drops of dilute acetic acid, when the turbidity, if due to phosphates, will disappear.

Nitric acid is often used in place of acetic acid in these tests. In case nitric acid is used ordinarily 1-2 drops is sufficient.

6. Acetic Acid and Potassium Ferrocyanide Test.—To 5 c.c. of urine in a test-tube add 5-10 drops of acetic acid. Mix well and add potassium ferrocyanide *drop by drop*, until a precipitate forms.

7. Tanret's Test.—To 5 c.c. of urine in a test-tube add Tanret's reagent¹ drop by drop until a turbidity or precipitate forms. This is an exceedingly delicate test. Sometimes the urine is stratified upon the reagent as in Heller's or Roberts' ring tests.

8. Sodium Chloride and Acetic Acid Test.—Mix two volumes of urine and one volume of a saturated solution of sodium chloride in a test-tube, acidify with acetic acid and heat to boiling. The production of a cloudiness or the formation of a precipitate indicates the presence of albumin. The resin acids may interfere here as in the ordinary coagulation test (page 292) but they may be easily differentiated from albumin by means of their solubility in alcohol.

GLOBULIN.

Serum globulin is not a constituent of normal urine but frequently occurs in the urine under pathological conditions and is ordinarily associated with serum albumin. In albuminuria globulin in varying amounts often accompanies the albumin, and the clinical significance of the two is very similar. Under certain conditions globulin may occur in the urine unaccompanied by albumin.

EXPERIMENTS.

Globulin will respond to all the tests just outlined under Albumin. If it is desirable to differentiate between albumin

¹ Tanret's reagent is prepared as follows: Dissolve 1.35 gram of mercuric chloride in 25 c.c. of water, add to this solution 3.32 grams of potassium iodide dissolved in 25 c.c. of water, then make the total solution up to 60 c.c. with water and add 20 c.c. of glacial acid to the mixture.

and globulin in any urine the following processes may be employed:

1. **Saturation With Magnesium Sulphate.**—Place 25 c.c. of neutral urine in a small beaker and add pulverized magnesium sulphate *in substance* to the point of saturation. If the proteid present is globulin it will precipitate at this point. If no precipitate is produced acidify the saturated solution with acetic acid and warm gently. Albumin will be precipitated if present.

The above procedure may be used to separate globulin and albumin if present in the same urine. To do this filter off the globulin after it has been precipitated by the magnesium sulphate, then acidify the clear solution and warm gently as directed. Note the formation of the albumin precipitate.

2. **Half-Saturation With Ammonium Sulphate.**—Place 25 c.c. of neutral urine in a small beaker and add an equal volume of a saturated solution of ammonium sulphate. Globulin, if present, will be precipitated. If no precipitate forms add ammonium sulphate *in substance* to the point of saturation. If albumin is present it will be precipitated upon saturation of the solution as just indicated. This method may also be used to separate globulin and albumin when they occur in the same urine.

Frequently in urine which contains a large amount of urates a precipitate of ammonium urate may occur when the ammonium sulphate solution is added to the urine. This urate precipitate should not be confounded with the precipitate due to globulin. The two precipitates may be differentiated by means of the fact that the urate precipitate ordinarily appears only after the lapse of several minutes whereas the globulin generally precipitates at once.

PROTEOSE AND PEPTONE.

Proteoses, particularly deuterio-proteose and hetero-proteose, have frequently been found in the urine under various pathological conditions such as diphtheria, pneumonia, intestinal

ulcer, carcinoma, dermatitis, osteomalacia, atrophy of the kidneys and in sarcomata of the bones of the trunk. "Bence-Jones' proteid," a proteose-like substance, is of interest in this connection and its appearance in the urine is believed to be of great diagnostic importance in cases of multiple myeloma or myelogenic osteosarcoma. By some investigators this proteid is held to be a variety of hetero-proteose whereas others claim that it possesses albumin characteristics.

Peptone certainly occurs much less frequently as a constituent of the urine than does proteose, in fact most investigators seriously question its presence under any conditions. There are many instances of peptonuria cited in the early literature but because of the uncertainty in the conception of what really constituted a peptone it is probable that in many cases of so-called peptonuria the proteid present was really proteose.

EXPERIMENTS.

1. **Boiling Test.**—Make the ordinary coagulation test according to the directions given under Albumin, page 292. If no coagulable proteid is found allow the boiled urine to stand and note the gradual appearance, in the cooled fluid, of a flaky precipitate of proteose. This is a crude test and should never be relied upon.

2. **Schulte's Method.**—Acidify 50 c.c. of urine with dilute acetic acid and filter off any precipitate of nucleo-proteid which may form. Now test a few cubic centimeters of the urine for coagulable proteid, by tests 2 and 5 under Albumin, pp. 291–292. If coagulable proteid is present remove it by coagulation and filtration before proceeding. Introduce 25 c.c. of the urine, freed from coagulable proteid, into 150 c.c. of absolute alcohol and allow it to stand for 12–24 hours. Decant the supernatant fluid and dissolve the precipitate in a small amount of hot water. Now filter this solution, and after testing again for nucleo-proteid with *very dilute* acetic acid, try the biuret test. If this test is positive the presence of proteose is indicated.¹

¹ If it is considered desirable to test for peptone the proteose may be removed by saturation with $(\text{NH}_4)_2\text{SO}_4$ according to the directions given on page 59 and the filtrate tested for peptone by the biuret test.

Urobilin does not ordinarily interfere with this test since it is almost entirely dissolved by the absolute alcohol when the proteose is precipitated.

3. **v. Aldor's Method.**—Acidify 10 c.c. of urine with hydrochloric acid, add phosphotungstic acid until no more precipitate forms and centrifugate¹ the solution. Decant the supernatant fluid, add some absolute alcohol to the precipitate and centrifugate again. This washing with alcohol is intended to remove the urobilin and hence should be continued so long as the alcohol exhibits any coloration whatever. Now suspend the precipitate in water and add potassium hydroxide to bring it into solution. At this point the solution may be blue in color in which case decolorization may be secured by gently heating. Apply the biuret test to the *cool* solution. A positive biuret test indicates the presence of proteoses.

4. **Detection of "Bence-Jones' Proteid."**—Heat the suspected urine very gently, carefully noting the temperature. At as low a temperature as 40° C. a turbidity may be observed and as the temperature is raised to about 60° C. a flocculent precipitate forms and clings to the sides of the test-tube. If the urine is now acidified *very slightly* with acetic acid and the temperature further raised to 100° C. the precipitate at least partly disappears; it will return upon cooling the tube.

This property of precipitating at so low a temperature and of dissolving at a higher temperature is typical of "Bence-Jones' proteid" and may be used to differentiate it from all other forms of proteid material occurring in the urine.

NUCLEO-PROTEID.

There has been considerable controversy as to the proper classification for the proteid body which forms the "nubecula" of normal urine. By different investigators it has been called *mucin*, *mucoid*, *phospho-proteid*, *nucleo-albumin* and *nucleo-proteid*. Of course, according to the modern acceptation of

¹ If not convenient to use a centrifuge the precipitate may be filtered off and washed on the filter paper with alcohol.

the meanings of these terms they cannot be synonymous. Mucin and mucoid are glucoproteids and hence contain no phosphorus (see p. 61), whereas phospho-proteids, nucleo-albumins and nucleo-proteids are phosphorized bodies. It may possibly be that both these forms of proteid, *i. e.*, the glucoproteid and the phosphorized type, occur in the urine under certain conditions (see page 264). In this connection we will use the term *nucleo-proteid*. The pathological conditions under which the content of nucleo-proteid is increased includes all affections of the urinary passages and in particular pyelitis, nephritis and inflammation of the bladder.

EXPERIMENTS.

1. **Detection of Nucleo-proteid.**—Place 10 c.c. of urine in a small beaker, dilute it with three volumes of water, to prevent precipitation of urates, and make the reaction *very strongly* acid with acetic acid. If the urine becomes turbid it is an indication that nucleo-proteid is present.

If the urine under examination contains albumin the greater portion of this substance should be removed by boiling the urine before testing it for the presence of nucleo-proteid.

2. **Ott's Precipitation Test.**—Mix 25 c.c. of the urine with an equal volume of a saturated solution of sodium chloride and slowly add Almén's reagent.¹ In the presence of nucleo-proteid a voluminous precipitate forms.

BLOOD.

The pathological conditions in which blood occurs in the urine may be classified under the two divisions *hæmaturia* and *hæmoglobinuria*. In *hæmaturia* we are able to detect not only the hæmoglobin but the unruptured corpuscles as well, whereas in *hæmoglobinuria* the pigment alone is present. *Hæmaturia* is brought about through blood passing into the urine because of some lesion of the kidney or of the urinary tract below the

¹ Dissolve 5 grams of tannin in 240 c.c. of 50 per cent alcohol and add 10 c.c. of 25 per cent acetic acid.

kidney. Hæmoglobinuria is brought about through hæmolysis, *i. e.*, the rupturing of the stroma of the erythrocyte and the liberation of the hæmoglobin. This may occur in scurvy, typhus, pyemia, purpura and in other diseases. It may also occur as the result of a burn covering a considerable area of the body, or may be brought about through the action of certain poisons or by the injection of various substances having the power of dissolving the erythrocytes. Transfusion of blood may also cause hæmoglobinuria.

EXPERIMENTS.

1. **Heller's Test.**—Render 10 c.c. of urine strongly alkaline with potassium hydroxide solution and heat to boiling. Upon allowing the heated urine to stand a precipitate of phosphates, colored red by the contained hæmatin, is formed. It is ordinarily well to make a "control" experiment using normal urine, before coming to a final decision.

Certain substances such as cascara sagrada, rhubarb, santonin, and senna cause the urine to give a similar reaction. Reactions due to such substances may be differentiated from the true blood reaction by the fact that both the precipitate and the pigment of the former reaction disappear when treated with acetic acid, whereas if the color is due to hæmatin the acid will only dissolve the precipitate of phosphates and leave the pigment undissolved.

2. **Teichmann's Hæmin Test.**—Place a small drop of the suspected urine or a small amount of the moist sediment on a microscopic slide, add a minute grain of NaCl and *carefully* evaporate to *dryness* over a *low* flame. Put a cover glass in place, run underneath it a drop of glacial acetic acid and warm gently until the formation of gas bubbles is observed. Cool the preparation, examine under the microscope and compare the form of the crystals with those reproduced in Figs. 58 and 59, page 164.

3. **Heller-Teichmann Reaction.**—Produce the pigmented precipitate according to directions given in Heller's test on p.

298. If there is a copious precipitate of phosphates and but little pigment the phosphates may be dissolved by treatment with acetic acid and the residue used in the formation of the hæmin crystals according to directions in Experiment 2, p. 298.

4. **Zeynek and Nencki's Hæmin Test.**—To 10 c.c. of the urine under examination add acetone until no more precipitate forms. Filter off the precipitate and extract it with 10 c.c. of acetone rendered acid with 2–3 drops of hydrochloric acid. Place a drop of the resulting colored extract on a slide, immediately place a cover glass in position and examine under the microscope. Compare the form of the crystals with those shown in Figs. 58 and 59, page 164. Hæmin crystals produced by this manipulation are sometimes very minute, thus rendering it difficult to determine the exact form of the crystal.

5. **Schalhjew's Hæmin Test.**—Place 20 c.c. of glacial acetic acid in a small beaker and heat to 80° C. Add 5 c.c. of the urine under examination, raise the temperature to 80° C. and stand the mixture aside to cool. Examine the crystals under the microscope and compare them with those shown in Figs. 58 and 59, page 164.

6. **Guaïac Test.**—Place 5 c.c. of urine in a test-tube and by means of a pipette introduce a freshly prepared alcoholic solution of guaïac into the fluid until a turbidity results; then add old turpentine or hydrogen peroxide, drop by drop, until a blue color is obtained. This is a very delicate test when properly performed. Buckmaster has recently suggested the use of guaiaconic acid instead of the solution of guaïac. See discussion on page 158 and test on page 163.

7. **Spectroscopic Examination.**—Submit the urine to a spectroscopic examination according to the directions given on page 169 looking especially for the absorption-bands of oxy-hæmoglobin and methæmoglobin (see Absorption Spectra, Plate I.).

BILE.

Both the pigments and the acids of the bile may be detected in the urine under certain pathological conditions. Of the pigments, bilirubin is the only one which has been positively identified in fresh urine; the other pigments, when present, are probably derived from the bilirubin. A urine containing bile may be yellowish-green to brown in color and when shaken foams readily. The staining of the various tissues of the body through the absorption of bile due to occlusion of the bile duct causes a condition known as icterus or jaundice. Bile is always present in the urine under such conditions unless the amount of bile reaching the tissues is extremely small.

EXPERIMENTS.

Tests for Bile Pigments.

1. **Gmelin's Test.**—To about 5 c.c. of *concentrated* nitric acid in a test-tube add an equal volume of urine *carefully* so that the two fluids do not mix. At the point of contact note the various colored rings, *green, blue, violet, red and reddish-yellow.*

2. **Rosenbach's Modification of Gmelin's Test.**—Filter 5 c.c. of urine through a small filter paper. Introduce a drop of *concentrated* nitric acid into the cone of the paper and observe the succession of colors as given in Gmelin's test.

3. **Huppert's Reaction.**—Thoroughly shake equal volumes of urine and milk of lime in a test-tube. The pigments unite with the calcium and are precipitated. Filter off the precipitate, wash it with water and transfer to a small beaker. Add alcohol acidified slightly with hydrochloric acid and warm upon a water-bath until the solution becomes colored an emerald green.

4. **Hammarsten's Reaction.**—To about 5 c.c. of Hammarsten's reagent¹ in a small evaporating dish add a few drops

¹Hammarsten's reagent is made by mixing 1 volume of 25 per cent nitric acid and 19 volumes of 25 per cent hydrochloric acid and then adding 1 volume of this acid mixture to 4 volumes of 95 per cent alcohol.

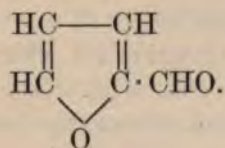
of urine. A green color is produced. If more of the reagent is now added the play of colors as noted in Gmelin's test may be obtained.

5. **Smith's Test.**—To 2–3 c.c. of urine in a test-tube add *carefully* about 5 c.c. of dilute tincture of iodine (1:10) so that the fluids do not mix. A green ring is observed at the point of contact.

Tests for Bile Acids.

1. **Pettenkofer's Test.**—To 5 c.c. of urine in a test-tube add 5 drops of a 5 per cent solution of saccharose. Now incline the tube, run about 2–3 c.c. of concentrated sulphuric acid *carefully* down the side and note the *red* ring at the point of contact. Upon slightly agitating the contents of the tube the whole solution gradually assumes a *reddish* color. As the tube becomes warm, it should be cooled in running water in order that the temperature may not rise above 70° C.

2. **Mylius's Modification of Pettenkofer's Test.**—To approximately 5 c.c. of urine in a test-tube add 3 drops of a very dilute (1:1,000) aqueous solution of furfural,



Now incline the tube, run about 2–3 c.c. of concentrated sulphuric acid *carefully* down the side and note the *red* ring as above. In this case also, upon shaking the tube, the whole solution is colored red. Keep the temperature below 70° C. as before.

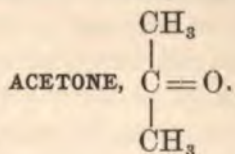
3. **Neukomm's Modification of Pettenkofer's Test.**—To a few drops of urine in an evaporating dish add a trace of a dilute saccharose solution and one or more drops of dilute sulphuric acid. Evaporate on a water-bath and observe the development of a *violet* color at the edge of the evaporating mixture. Discontinue the evaporation as soon as the color is observed.

4. **v. Udránsky's Test.**—To 5 c.c. of urine in a test-tube add 3-4 drops of a very dilute (1:1,000) aqueous solution of furfural. Place the thumb over the top of the tube and shake until a thick foam is formed. By means of a small pipette add 2-3 drops of concentrated sulphuric acid to the foam and observe the *dark pink* coloration produced.

5. **Salkowski's Test.**—Render 5 c.c. of urine alkaline with a few drops of a 10 per cent sodium carbonate solution and add a 10 per cent solution of calcium chloride, drop by drop, until the supernatant fluid exhibits the normal urinary color when the contents of the test-tube are thoroughly mixed. Filter off the precipitate, and after washing it, place it in a second tube with 95 per cent alcohol. Acidify the alcohol with hydrochloric acid and, if necessary, shake the tube to bring the precipitate into solution. Heat the solution to boiling and observe the appearance of a green color which changes through blue and violet to red; if no bile is present the solution does not undergo any color change. This test will frequently exhibit greater delicacy than Gmelin's test.

6. **Hay's Test.**—Cool about 10 c.c. of urine in a test-tube to 17° C. or lower, and sprinkle a little finely pulverized sulphur upon the surface of the fluid. The presence of bile acids is indicated if the sulphur sinks to the bottom of the liquid, the rapidity with which the sulphur sinks depending upon the amount of bile acids present in the urine. The test is said to react with bile acids when the latter are present in the proportion 1:120,000.

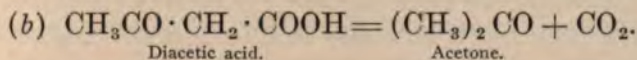
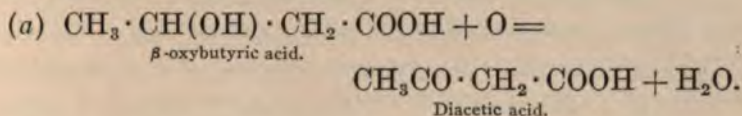
Some investigators claim that it is impossible to differentiate between bile acids and bile pigments by this test.



It was formerly very generally believed that acetone appeared

in the urine under pathological conditions because of increased proteid decomposition. It is now generally thought that, in man, the output of acetone arises principally from the breaking down of fatty tissues or fatty foods within the organism. The quantity of acetone eliminated has been shown to increase when the subject is fed an abundance of fat-containing food as well as during fasting, whereas a replacement of the fat with carbohydrates is followed by a marked decrease in the acetone excretion. Conditions are different with certain of the lower animals. With the dog, for instance, the output of acetone is not diminished when the animal is fed upon a carbohydrate diet, is *decreased* during fasting and increased when the animal is fed upon a diet of meat.

Acetone and the closely related bodies, β -oxybutyric acid and diacetic acid, are generally classified as the *acetone bodies*. They are all associated with a deranged metabolic function and may appear in the urine together or separately, depending upon the conditions. Acetone and diacetic acid may occur alone in the urine but β -oxybutyric acid is never found except in conjunction with one or the other of these bodies. Acetone and diacetic acid arise chiefly from the oxidation of β -oxybutyric acid. The relation existing between these three bodies is shown in the following reactions:



Acetone, chemically considered, is a ketone, *di-methyl ketone*. When pure it is a liquid which possesses a characteristic aromatic fruit-like odor, boils at 56–57° C. and is miscible with water, alcohol or ether in all proportions. Acetone is a *physiological* as well as a pathological constituent of the urine and under normal conditions the daily output is about 0.01–0.03 gram.

Pathologically, the elimination of acetone is often greatly increased and at such times a condition of *acetonuria* is said to exist. This pathological acetonuria may accompany diabetes mellitus, scarlet fever, typhoid fever, pneumonia, nephritis, phosphorus poisoning, grave anæmias, fasting and a deranged digestive function; it also frequently accompanies auto-intoxication and chloroform anæsthesia. The types of acetonuria most frequently met with are those noted in febrile conditions and in advanced cases of diabetes mellitus.

EXPERIMENTS.

1. **Isolation from the Urine.**—In order to facilitate the detection of acetone in the urine, the specimen under examination should be distilled and the tests as given below applied to the resulting distillate. If it is not convenient to distil the urine the tests may be conducted upon the undistilled fluid. To obtain an acetone distillate proceed as follows: Place 100–250 c.c. of urine in a distillation flask or retort and render it acid with acetic acid. Collect about one-third of the original volume of fluid as a distillate, add 5 drops of 10 per cent hydrochloric acid and redistil about one-half of this volume. With this final distillate conduct the tests as given below.

2. **Gunning's Iodoform Test.**—To about 5 c.c. of the urine or distillate in a test-tube add a few drops of Lugol's solution¹ or ordinary iodine solution (I in KI) and enough NH_4OH to form a black precipitate (nitrogen iodide). Allow the tube to stand (the length of time depending upon the content of acetone in the fluid under examination) and note the formation of a yellowish sediment consisting of iodoform. Examine the sediment under the microscope and compare the form of the crystals with those shown in Fig. 6, p. 21. If the crystals are not well formed recrystallize them from ether and examine again. The crystals of iodoform should not be confounded with those of stellar phosphate (Fig. 76, p. 193) which may be formed in

¹Lugol's solution may be prepared by dissolving 5 grams of iodine and 10 grams of potassium iodide in 100 c.c. of distilled water.

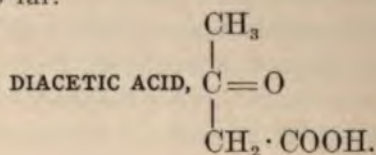
this test, particularly if made upon the undistilled urine. This test is preferable to Lieben's test (4) since no substance other than acetone will produce iodoform when treated according to the directions for this test; both alcohol and aldehyde yield iodoform when tested by Lieben's test.

Gunning's test is rather the most satisfactory test yet suggested for the detection of acetone, and may be used with good results even upon the undistilled urine. In some instances where the amount of acetone present is very small it is necessary to allow the tube to stand 24 hours before making the examination for iodoform crystals. This test serves to detect acetone when present in the ratio 1 : 100,000.

3. **Legal's Test.**—Introduce about 5 c.c. of the urine or distillate into a test-tube, add a few drops of a freshly prepared aqueous solution of sodium nitro-prusside and render the mixture alkaline with potassium hydroxide. A ruby red color, due to creatinin, a normal urinary constituent, is produced (see Weyl's test, p. 252). Add an excess of acetic acid and if acetone is present the red color will be intensified, whereas in the absence of acetone a yellow color will result. Make a control test upon normal urine to show that this is so. A similar red color may be produced by paracresol in urines containing no acetone.

4. **Lieben's Test.**—Introduce 5 c.c. of the urine or distillate into a test-tube, render it alkaline with potassium hydroxide and add 1-2 c.c. of iodine solution, drop by drop. If acetone is present a yellowish precipitate of iodoform will be produced. Identify the iodoform by means of its characteristic odor and its typical crystalline form (see Fig. 6, p. 21). While fully as delicate as Gunning's test (2) this test is not as accurate, since by means of the procedure involved, either alcohol or aldehyde will yield a precipitate of iodoform. This test is especially liable to lead to erroneous deductions when urines from the advanced stages of diabetes are under examination, because of the presence of alcohol formed from the sugar through fermentative processes.

5. **Reynolds-Gunning Test.**—This test depends upon the solubility of mercuric oxide in acetone and is performed as follows: To 5 c.c. of the urine or distillate add a few drops of mercuric chloride, render the solution alkaline with potassium hydroxide and add an equal volume of 95 per cent alcohol. Shake thoroughly in order to bring the major portion of the mercuric oxide into solution and filter. Render the *clear* filtrate faintly acid with hydrochloric acid and stratify some ammonium sulphide, $(\text{NH}_4)_2\text{S}$, upon this acid solution. At the zone of contact a blackish-gray ring of precipitated mercuric sulphide, HgS , will form. Aldehyde also responds to this test. Aldehyde, however, has never been detected in the urine and could only be present in this instance if the acidified urine was distilled too far.



Diacetic or acetoacetic acid occurs in the urine only under pathological conditions and is rarely found except associated with acetone. It is formed from β -oxybutyric acid, another of the *acetone bodies*, and upon decomposition yields acetone and carbon dioxide. Diaceturia occurs ordinarily under the same conditions as the pathological acetonuria, *i. e.*, in fevers, diabetes, etc. (see p. 304). If very little diacetic acid is formed it may all be transformed into acetone, whereas if a larger quantity is produced both acetone and diacetic acid may be present in the urine. Diaceturia is most frequently observed in children, especially accompanying fevers and digestive disorders; it is perhaps less frequently observed in adults, but when present, particularly in fevers and diabetes, it is frequently followed by fatal coma.

Diacetic acid is a colorless liquid which is miscible with water, alcohol, and ether, in all proportions. It differs from acetone in giving a violet-red or Bordeaux-red color with a dilute solution of ferric chloride.

EXPERIMENTS.

1. **Gerhardt's Test.**—To 5 c.c. of urine in a test-tube add ferric chloride solution, drop by drop, until no more precipitate forms. In the presence of diacetic acid a Bordeaux-red color is produced; this color may be somewhat masked by the precipitate of ferric phosphate, in which case the fluid should be filtered.

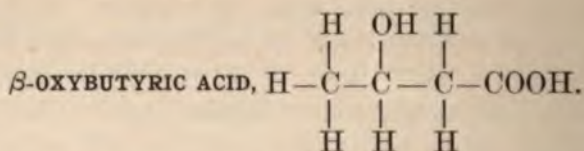
A positive result from the above manipulation simply indicates the *possible* presence of diacetic acid. Before making a final decision regarding the presence of this body make the two following control experiments:

(a) Place 5 c.c. of urine in a test-tube and boil it vigorously for 3–5 minutes. Cool the tube and, with the boiled urine, make the test as given above. As has been already stated, diacetic acid yields acetone upon decomposition and acetone does *not* give a Bordeaux-red color with ferric chloride. By boiling as indicated above therefore, any diacetic acid present would be decomposed into acetone and carbon dioxide and the test upon the resulting fluid would be negative. If positive the color is due to the presence of bodies other than diacetic acid.

(b) Place 5 c.c. of urine in a test-tube, acidify with H_2SO_4 , to free diacetic acid from its salts, and *carefully* extract the mixture with ether by shaking. If diacetic acid is present it will be extracted by the ether. Now remove the ethereal solution and add to it an equal volume of *dilute* ferric chloride; diacetic acid is indicated by the production of the characteristic Bordeaux-red color. This color disappears spontaneously in 24–48 hours. Such substances as antipyrin, kairin, phenacetin, salicylic acid, salicylates, sodium acetate, sulphocyanides and thallin yield a similar red color under these conditions, but when due to the presence of any of these substances the color does not disappear spontaneously but may remain permanent for days. Many of these disturbing substances are soluble in benzene or chloroform and may be removed from the urine by this means before extracting with ether as above. Diacetic acid is insoluble in benzene or chloroform.

2. **Arnold-Lipliawsky Reaction.**—This reaction is somewhat more delicate than Gerhardt's test (1) and serves to detect diacetic acid when present in the proportion 1 : 25,000. It is also negative toward acetone, β -oxybutyric acid and the interfering drugs mentioned as causing erroneous deductions in the application of Gerhardt's test. If the urine under examination is highly pigmented it should be partly decolorized by means of animal charcoal before applying the test as indicated below.

Place 5 c.c. of the urine under examination and an equal volume of the Arnold-Lipliawsky reagent¹ in a test-tube, add a few drops of concentrated ammonia and shake the tube vigorously. Note the production of a brick-red color. Take 1–2 c.c. of this colored solution, add 10–20 c.c. of hydrochloric acid (sp. gr. 1.19), 3 c.c. of chloroform and 2–4 drops of ferric chloride solution and carefully mix the fluids *without shaking*. Diacetic acid is indicated by the chloroform assuming a violet or blue color; if diacetic acid is absent the color may be yellow or light red.



This acid does not occur as a normal constituent of urine but is found only under pathological conditions and then always in conjunction with either acetone or diacetic acid. Either of these bodies may be formed from β -oxybutyric acid under proper conditions. It is present in especially large

¹This reagent consists of two definite solutions which are ordinarily preserved separately and mixed just before using. The two solutions are prepared as follows:

(a) One per cent aqueous solution of potassium nitrite.

(b) One gram of *p*-amino-acetophenon dissolved in 100 c.c. of distilled water and enough hydrochloric acid (about 2 c.c.) added, drop by drop, to cause the solution, which is at first yellow, to become entirely colorless. An excess of acid must be avoided.

Before using, *a* and *b* are mixed in the ratio 1 : 2.

amount in severe cases of diabetes and has also been detected in digestive disturbances, continued fevers, scurvy, measles and in starvation. It is probable that, in man, β -oxybutyric acid, in common with acetone and diacetic acid, arises principally from the breaking down of fatty tissues within the organism. The condition in which large amounts of acetone and diacetic acid, and in severe cases β -oxybutyric acid also, are excreted in the urine is known as "acidosis." In diabetes the deranged metabolic conditions cause the production of great quantities of these substances which lead to an acid intoxication and ultimately to diabetic coma.

Ordinarily β -oxybutyric acid is an odorless, transparent syrup, which is lævorotatory and easily soluble in water, alcohol and ether; it may be obtained in crystalline form.

EXPERIMENTS.

1. **Polariscopic Examination.**—Subject some of the urine (free from proteid) to the ordinary fermentation test (see page 288). This will remove dextrose and lævulose, which would interfere with the polariscopic test. Now examine the fermented fluid in the polariscope and if it is lævorotatory the presence of β -oxybutyric acid is indicated. This test is not absolutely reliable, however, since conjugate glucuronates are also lævorotatory after fermentation.

2. **Külz's Test.**—Evaporate the urine, after fermenting it as indicated in the last test, to a syrup, add an equal volume of concentrated sulphuric acid and distil the mixture directly without cooling. Under these conditions α -crotonic acid is formed and is present in the distillate. Allow the distillate to cool slowly and note the formation of crystals of α -crotonic acid which are soluble in ether and melt at 72°C . In case very slight traces of β -oxybutyric acid be present in the urine under examination the amount of α -crotonic acid formed may be too small to yield a crystalline product. In this event the distillate should be extracted with ether, the ethereal extract evaporated and the residue washed with water. Under these

conditions the impurities will be removed and the α -crotonic acid will remain behind as a residue. The melting-point of this residue may then be determined.

CONJUGATE GLYCURONATES.

Glycuronic acid does not occur free in the urine but is found, for the most part, in combination with phenol. Much smaller quantities are excreted in combination with indoxyl and skatoxyl. The total content of conjugate glycuronates seldom exceeds 0.004 per cent under normal conditions. The output may be very greatly increased as the result of the administration of antipyrin, borneol, camphor, chloral, menthol, morphine, naphthol, turpentine, etc. The glycuronates as a group are lævorotatory, whereas glycuronic acid is dextro-rotatory. Most of the glycuronates reduce alkaline metallic oxides and so introduce an error in the examination of urine for sugar. Conjugate glycuronates often occur associated with dextrose in glycosuria, diabetes mellitus and in some other disorders. As a class the glycuronates are non-fermentable.

EXPERIMENTS.

1. **Fermentation-Reduction Test.**—Test the urine by Fehling's test. If there is reduction try Barfoed's test. If negative this indicates the absence of dextrose. A negative fermentation test would now indicate the presence of conjugate glycuronates (or lactose in rare cases).

If dextrose is present in the urine tested for glycuronates the urine must first be subjected to a polariscopic examination, then fermented and a second polariscopic examination made. The sugar being dextro-rotatory and fermentable and the glycuronates being lævorotatory and non-fermentable the second polariscopic test will show a lævorotation indicative of conjugate glycuronates.

2. **Tollens' Reaction.**—Make this test according to directions given under Pentoses, page 311.

PENTOSES.

We have two distinct types of pentosuria, *i. e.*, *alimentary pentosuria*, resulting from the ingestion of large quantities of pentose-rich vegetables such as prunes, cherries, grapes or plums, and fruit juices, in which condition the pentoses appear only *temporarily* in the urine; and the *chronic* form of pentosuria, in which the output of pentoses bears no relation whatever to the quantity and nature of the pentose content of the food eaten. In occurring in these two forms, pentosuria resembles glycosuria (see page 283), but it is definitely known that pentosuria bears no relation to diabetes mellitus and there is no generally accepted theory to account for the occurrence of the chronic form of pentosuria. The pentose detected most frequently in the urine is arabinose, the inactive form generally occurring in chronic pentosuria and the *lævo*-rotatory variety occurring in the alimentary type of the disorder.

EXPERIMENTS.

1. **Tollens' Reaction.**—To equal volumes of urine and hydrochloric acid (sp. gr. 1.09) add a little phloroglucin and heat the mixture on a boiling water-bath. Pentose, galactose, *lævulose* or glycuronic acid will be indicated by the appearance of a red color. To differentiate between these bodies examine by the spectroscope and look for the absorption band between D and E given by pentoses and glycuronic acid, and then differentiate between the two latter bodies by the melting-points of their osazons.

2. **Orcin Test.**—Place equal volumes of urine and hydrochloric acid (sp. gr. 1.09) in a test-tube, add a small amount of orcin, and heat the mixture to boiling. Color changes from red, through reddish-blue to green will be noted. When the solution becomes green it should be shaken in a separatory funnel with a little amyl alcohol, and the alcoholic extract examined spectroscopically. An absorption band between C and D will be observed.

FAT.

When fat finds its way into the urine through a lesion which brings some portion of the urinary passages into communication with the lymphatic system a condition known as *chyluria* is established. The turbid or milky appearance of such urine is due to its content of chyle. This disease is encountered most frequently in tropical countries, but is not entirely unknown in more temperate climates. Albumin is a constant constituent of the urine in *chyluria*. Upon shaking a chylous urine with ether the fat is dissolved by the ether and the urine becomes clearer or entirely clear.

HÆMATOPORPHYRIN.

Urine containing this body is occasionally met with in various diseases but more frequently after the use of quinine, tetronal, trional and especially sulphonal. Such urines ordinarily possess a reddish tint, the depth of color varying greatly under different conditions.

EXPERIMENTS.

1. **Spectroscopic Examination.**—To 100 c.c. of urine add about 20 c.c. of a 10 per cent solution of KOH or NH_4OH . The precipitate which forms consists principally of earthy phosphates to which the hæmatoporphyrin adheres and is carried down. Filter off the precipitate, wash it and transfer to a flask and warm with alcohol acidified with hydrochloric acid. By this process the hæmatoporphyrin is dissolved and on filtering will be found in the filtrate and may be identified by means of the spectroscope (see page 173, and Absorption Spectra, Plate II).

2. **Acetic Acid Test.**—To 100 c.c. of urine add 5 c.c. of glacial acetic acid and allow the mixture to stand 48 hours. Hæmatoporphyrin deposits in the form of a precipitate.

LACTOSE.

Lactose is rarely found in the urine except as it is excreted by women during pregnancy, during the nursing period or soon after weaning. It is rather difficult to show the presence of lactose in the urine in a satisfactory manner, since the formation of the characteristic lactosazon is not attended with any great measure of success under these conditions. It is, however, comparatively easy to show that it is not dextrose, for, while it responds to reduction tests, it does not ferment with *pure* yeast and does not give a dextrosazon. An absolutely conclusive test, of course, is the isolation of the lactose in crystalline form (Fig. 75, p. 189) from the urine.

EXPERIMENTS.

1. **Rubner's Test.**—To 10 c.c. of urine in a small beaker add some plumbic acetate, in substance, heat to boiling and add NH_4OH until no more precipitate is dissolved. In the presence of lactose a brick-red or rose-red color develops, whereas dextrose gives a coffee-brown color, maltose a light yellow color and lævulose no color at all under the same conditions.

2. **Compound Test.**—Try the phenylhydrazin test, the fermentation test and Barfoed's test according to directions given under Dextrose, pages 283, 288 and 289. If these are negative, try Nylander's test, page 288. If this last test is positive, the presence of lactose is indicated.

LÆVULOSE.

Diabetic urine frequently possesses the power of rotating the plane of polarized light to the left, thus indicating the presence of a lævorotatory substance. This lævorotation is sometimes due to the presence of lævulose, although not necessarily confined to this carbohydrate, since conjugate glycuronates and β -oxybutyric acid, two other lævorotatory bodies,

are frequently found in the urine of diabetics. Lævulose is invariably accompanied by dextrose in diabetic urine, but *lævulosuria* has been observed as a separate anomaly. The presence of lævulose may be inferred when the percentage of sugar, as determined by the titration method, is greater than the percentage indicated by the polariscopic examination.

EXPERIMENTS.

1. **Seliwanoff's Reaction.**—If a solution of resorcin in dilute HCl (1 volume of concentrated HCl to 2 volumes of H_2O) be warmed with an equal volume of a urine containing lævulose, the liquid will become red and a precipitate will separate. The precipitate may be dissolved in alcohol to which it will impart a striking red color.

2. **Phenylhydrazin Test.**—Make the test according to directions under Dextrose, 1 page 283.

3. **Polariscopic Examination.**—A simple polariscopic examination, when taken in connection with other ordinary tests, will furnish the requisite data regarding the presence of lævulose, provided lævulose is not accompanied by other lævorotatory substances, such as conjugate glycuronates and β -oxybutyric acid.

INOSIT.

Inosit occasionally occurs in the urine in albuminuria, diabetes mellitus and diabetes insipidus. It is claimed also that copious water-drinking causes this body to appear in the urine. By some investigators inosit is believed to occur in traces in normal urine.

EXPERIMENT.

1. **Detection of Inosit.**—Acidify the urine with concentrated nitric acid and evaporate nearly to dryness. Add a few drops of NH_4OH and a little $CaCl_2$ solution to the moist residue and evaporate the mixture to dryness. In the presence of inosit (0.001 gram) a bright red color is obtained.

LAILOSE.

This substance is *occasionally* found in the urine in severe cases of diabetes mellitus. By some investigators laiose is classed with the sugars. It resembles lævulose in that it has the property of reducing certain metallic oxides and is lævoro-tatory, but differs from lævulose in being amorphous, non-fermentable and in not possessing a sweet taste.

MELANINS.

These pigments never occur normally in the urine but are present under certain pathological conditions, their presence being especially associated with melanotic tumors. Ordinarily the freshly passed urine is clear, but upon exposure to the air the color deepens and may at the last be very dark brown or black in color. The pigment is probably present in the form of a chromogen or melanogen and upon coming in contact with the air oxidation occurs, causing the transformation of the melanogen into melanin and consequently the darkening of the urine.

It is claimed that melanuria is proof of the formation of a visceral melanotic growth. In many instances, without doubt, urines rich in indican have been wrongly taken as diagnostic proof of melanuria. The pigment melanin is sometimes mistaken for indigo and melanogen for indican. It is comparatively easy to differentiate between indigo and melanin through the solubility of the former in chloroform.

In rare cases melanin is found in urinary sediment in the form of fine amorphous granules.

EXPERIMENTS.

1. **Zeller's Test.**—To 50 c.c. of urine in a small beaker add an equal volume of bromine water. In the presence of melanin a yellow precipitate will form and will gradually darken in color, ultimately becoming black.

2. **von Jaksch-Pollak Reaction.**—Add a few drops of ferric chloride solution to 10 c.c. of urine in a test-tube and note the formation of a gray color. Upon the further addition of the chloride a dark precipitate forms, consisting of phosphates and adhering melanin. An excess of ferric chloride causes the precipitate to dissolve.

This is the most satisfactory test for the identification of melanin in the urine.

UROROSEIN.

This is a pigment which is not present in normal urine but may be detected in the urine of various diseases, such as pulmonary tuberculosis, typhoid fever, nephritis and stomach disorders. Urorosein, in common with various other pigments, does not occur preformed in the urine, but is present in the form of a chromogen, which is transformed into the pigment upon treatment with a mineral acid.

EXPERIMENTS.

1. **Robin's Reaction.**—Acidify 10 c.c. of urine with about 15 drops of concentrated hydrochloric acid. Upon allowing the acidified urine to stand, a rose-red color will appear if urorosein is present.

2. **Nencki and Sieber's Reaction.**—To 100 c.c. of urine in a beaker add 10 c.c. of 25 per cent sulphuric acid. Allow the acidified urine to stand and note the appearance of a rose-red color. The pigment may be separated by extraction with amyl alcohol.

UNKNOWN SUBSTANCES.

Ehrlich's Diazo Reaction.—Place equal volumes of urine and Ehrlich's diazobenzenesulphonic acid reagent¹ in a test-

¹Two separate solutions should be prepared and mixed in definite proportions when needed for use.

(a) Five grams of sodium nitrite dissolved in 1 liter of distilled water.

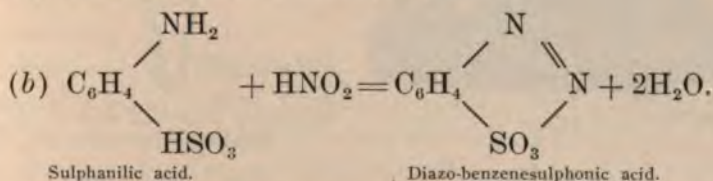
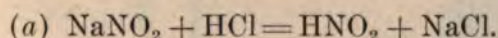
(b) Five grams of sulphanilic acid and 50 c.c. of hydrochloric acid in 1 liter of distilled water.

tube, mix thoroughly by shaking and quickly add ammonium hydroxide in excess. The test is positive if both the fluid and the foam assume a red color. If the tube is allowed to stand a precipitate forms, the upper portion of which exhibits a blue, green, greenish-black or violet color. Normal urine gives a brownish-yellow reaction with the above manipulation.

The exact nature of the substance or substances upon whose presence in the urine this reaction depends is not well understood. Some investigators claim that a positive reaction indicates an abnormal decomposition of proteid material, whereas others assume it to be due to an increased excretion of alloxypoteic acid, oxyproteic acid or uroferric acid.

The reaction may be taken as a metabolic symptom of certain disorders, which is of value diagnostically *only* when taken in connection with the other symptoms. The reaction appears principally in the urine in febrile disorders and in particular in the urine in typhoid fever, tuberculosis and measles. The reaction has also been obtained in the urine in various other disorders such as carcinoma, chronic rheumatism, diphtheria, erysipelas, pleurisy, pneumonia, scarlet fever, syphilis, typhus, etc. The administration of alcohol, chrysarobin, creosote, cresol, dionin, guaiacol, heroin, morphine, naphthalene, opium, phenol, tannic acid, etc., will also cause the urine to give a positive reaction.

The following chemical reactions take place in this test:



Sulphanilic acid.

Diazo-benzenesulphonic acid.

Solutions *a* and *b* should be preserved in well stoppered vessels and mixed in the proportion 1:50 when required. Green asserts that greater delicacy is secured by mixing the solutions in the proportion 1:100. The sodium nitrite deteriorates upon standing and becomes unfit for use in the course of a few weeks.

CHAPTER XIX.

URINE: ORGANIZED AND UNORGANIZED SEDIMENTS.

The data obtained from carefully conducted microscopical examinations of the sediment of certain pathological urines are of very great importance, diagnostically. Too little emphasis is sometimes placed upon the value of such findings.

FIG. 97.



THE PURDY ELECTRIC CENTRIFUGE.

FIG. 98.



SEDIMENT TUBE FOR THE PURDY
ELECTRIC CENTRIFUGE.

The sedimentary constituents may be divided into two classes, *i. e.*, *organized* and *unorganized*. The sediment is ordinarily collected for examination by means of the centrifuge (Fig. 97, above). An older method, and one still in

vogue in some quarters, is the so-called *gravity* method. This simply consists in placing the urine in a conical glass and allowing the sediment to settle. The collection of the sediment by means of the centrifuge, however, is much preferable, since the process of sedimentation may be accomplished by the use of this instrument in a few minutes, and far more perfectly, whereas when the other method is used it is frequently necessary to allow the urine to remain in the conical glass 12-24 hours before sufficient sediment can be secured for the microscopical examination.

(a) Unorganized Sediments.

Ammonium magnesium phosphate ("Triple phosphate").

Calcium oxalate.

Calcium carbonate.

Calcium phosphate.

Calcium sulphate.

Uric acid.

Urates.

Cystin.

Cholesterin.

Hippuric acid.

Leucin and tyrosin.

Hæmatoidin and bilirubin.

Magnesium phosphate.

Indigo.

Xanthin.

Melanin.

Ammonium Magnesium Phosphate ("Triple Phosphate").—Crystals of "triple phosphate" are a characteristic constituent of the sediment when alkaline fermentation of the urine has taken place either *before* or after being voided. They may even be detected in amphoteric or *slightly* acid urine provided the ammonium salts are present in large enough quantity. This substance may occur in the sediment in two forms, *i. e.*, prisms and the feathery type. The pris-

matic form of crystal (Fig. 96, p. 278) is the one most commonly observed in the sediment; the feathery form (Fig. 96, p. 278) predominates when the urine is made ammoniacal with ammonia.

The sediment of the urine in such disorders as are accompanied by a retention of urine in the lower urinary tract contains "triple phosphate" crystals as a characteristic constituent. The crystals are frequently abundant in the sediment during paraplegia, chronic cystitis, enlarged prostate and chronic pyelitis.

Calcium Oxalate.—Calcium oxalate is found in the urine in the form of at least two distinct types of crystals, *i. e.*, the *dumb-bell* type and the *octahedral* type (Fig. 99, below).

FIG. 99.



CALCIUM OXALATE. (Ogden.)

Either form may occur in the sediment of neutral, alkaline or acid urine, but both forms are found most frequently in urine having an acid reaction. Occasionally, in alkaline urine, the octahedral form is confounded with "triple phosphate" crystals. They may be differentiated from the phosphate crystals by the fact that they are insoluble in acetic acid.

The presence of calcium oxalate in the urine is not of itself a sign of any abnormality, since it is a constituent of normal urine. It is increased above the normal, however, in such pathological conditions as diabetes mellitus, in organic diseases of the liver and in various other conditions which are accompanied by a derangement of digestion or of the oxida-

tion mechanism, such as occurs in certain diseases of the heart and lungs.

Calcium Carbonate.—Calcium carbonate crystals form a typical constituent of the urine of herbivorous animals. They occur less frequently in human urine. The reaction of urine containing these crystals is nearly always alkaline, although they may occur in amphoteric or in *slightly* acid urine. It generally crystallizes in the form of granules, spherules or dumbbells (Fig. 100, below). The crystals of calcium carbonate may be differentiated from calcium oxalate by the fact that they dissolve in acetic acid with the evolution of carbon dioxide gas.

Calcium Phosphate (Stellar Phosphate).—Calcium phosphate may occur in the urine in three forms, *i. e.*, amorphous, granular or crystalline. The crystals of calcium phosphate

FIG. 100.



CALCIUM CARBONATE.

are ordinarily pointed, wedge-shaped formations which may occur as individual crystals or grouped together in more or less regularly formed rosettes (Fig. 76, p. 193). Acid sodium urate crystals (Fig. 102, p. 324) are often mistaken for crystals of calcium phosphate. We may differentiate between these

two crystalline forms by the fact that acetic acid will readily dissolve the phosphate, whereas the urate is much less soluble and when finally brought into solution and recrystallized one is frequently enabled to identify uric acid crystals which have been formed from the acid urate solution. The clinical significance of the occurrence of calcium phosphate crystals in the urinary sediment is similar to that of "triple phosphate" (see page 319).

Calcium Sulphate.—Crystals of calcium sulphate are of quite rare occurrence in the sediment of urine. Their presence seems to be limited in general to urines which are of a decided acid reaction. Ordinarily it crystallizes in the form of long, thin, colorless needles or prisms (Fig. 95, page 274) which may be mistaken for calcium phosphate crystals. There need be no confusion in this respect, however, since the sulphate crystals are insoluble in acetic acid which reagent readily dissolves the phosphate. As far as is known their occurrence as a constituent of urinary sediment is of very little clinical significance.

Uric Acid.—Uric acid forms a very common constituent of the sediment of urines which are acid in reaction. It occurs in more varied forms than any of the other crystalline sediments (Plate V, opposite page 247, and Fig. 101, page 323), some of the more common varieties of crystals being rhombic prisms, wedges, dumb-bells, whetstones, prismatic rosettes, irregular rectangular or hexagonal plates, etc. Crystals of pure uric acid are always colorless (Fig. 89, page 249) but the form occurring in urinary sediments is impure and under the microscope appears pigmented, the depth of color varying from light yellow to a dark reddish-brown according to the size and form of the crystal.

The presence of a considerable uric acid sediment does not, of necessity, indicate a pathological condition or a urine of increased uric acid content, since this substance very often occurs as a sediment in urines whose uric acid content is diminished from the normal merely as a result of changes in

reaction, etc. Pathologically, uric acid sediments occur in gout, acute febrile conditions, chronic interstitial nephritis, etc. If the microscopical examination is not conclusive, uric acid may be differentiated from other crystalline urinary sedi-

FIG. 101.



VARIOUS FORMS OF URIC ACID.

1, Rhombic plates; 2, whetstone forms; 3, 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosettes; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine.

ments from the fact that it is soluble in alkalis, alkali carbonates, boiling glycerin, concentrated sulphuric acid and in certain organic bases such as ethylamine and piperidin. It also responds to the murexid test (see page 249) and to Schiff's reaction (see page 249).

Urates.—The urate sediment may consist of a mixture of the urates of ammonium, calcium, magnesium, potassium and sodium. The ammonium urate may occur in neutral, alkaline or acid urine, whereas the other forms of urates are confined to the sediments of acid urines. Sodium urate occurs in sediments more abundantly than the other urates.

The urates of calcium, magnesium and potassium are amorphous in character, whereas the urate of ammonium is crystalline. Sodium urate may be either amorphous or crystalline. When crystalline it forms groups of fan-shaped clusters or colorless, prismatic needles (Fig. 102, below). Ammonium urate is ordinarily present in the sediment in the burr-like form of the "thorn-apple" crystal, *i. e.*, yellow or reddish-brown spheres, covered with sharp spicules or prisms (Plate VI, opposite page 324). The urates are all soluble in hydro-

FIG. 102.



ACID SODIUM URATE.

chloric acid or acetic acid and their acid solutions yield crystals of uric acid upon standing. They also respond to the murexid test. The clinical significance of urate sediments is very similar to that of uric acid. A considerable sediment of amorphous urates does not necessarily indicate a high uric acid content, but ordinarily signifies a concentrated urine having a very strong acidity.

Cystin.—Cystin is one of the rarer of the crystalline urinary sediments. It has been claimed that it occurs more often in the urine of men than of women. Cystin crystallizes in the form of thin, colorless, hexagonal plates (Fig. 32,

PLATE VI.



AMMONIUM URATE, SHOWING SPHERULES AND THORN-APPLE-SHAPED CRYSTALS.
(From Ogden, after Peyer.)

p. 76, and Fig. 103, below) which are insoluble in water, alcohol and acetic acid and soluble in mineral acids, alkalis and especially in ammonia. Cystin may be identified by burning it upon platinum foil under which condition it does not melt but yields a bluish-green flame.

FIG. 103.



CYSTIN. (Ogden.)

Cholesterin.—Cholesterin crystals have been but rarely detected in urinary sediments. When present they probably arise from a pathological condition of some portion of the urinary tract. Crystals of cholesterin have been found in the sediment in cystitis, pyelitis, chyluria and nephritis. Ordinarily it crystallizes in large regular and irregular colorless, transparent plates, some of which possess notched corners (Fig. 42, page 125). Frequently, instead of occurring in the sediment, it is found in the form of a film on the surface of the urine.

Hippuric Acid.—This is one of the rarer sediments of human urine. It deposits under conditions similar to those which govern the formation of uric acid sediments. The crystals, which are colorless needles or prisms (Fig. 92, page 256) when pure, are invariably pigmented in a manner similar to the uric acid crystals when observed in urinary sediment and because of this fact are frequently confounded with the rarer forms of uric acid. Hippuric acid may be differentiated from uric acid from the fact that it does not respond to the murexid test and is much more soluble in water and in ether. The detection of crystals of hippuric acid in the

urine has very little clinical significance, since its presence in the sediment depends in most instances very greatly upon the nature of the diet. It is particularly prone to occur in the sediment after the ingestion of certain fruits as well as after the ingestion of benzoic acid (see page 256).

Leucin and Tyrosin.—Leucin and tyrosin have frequently been detected in the urine, either in solution or as a sediment. Neither of them occurs in the urine ordinarily except in association with the other, *i. e.*, whenever leucin is detected it is more than probable that tyrosin accompanies it. They have been found pathologically in the urine in acute yellow atrophy of the liver, in acute phosphorus poisoning, in cirrhosis of the liver, in severe cases of typhoid fever and smallpox, and in leukæmia. In urinary sediments leucin ordinarily crystallizes in characteristic spherical masses which

show both radial and concentric striations and are highly refractive (Fig. 104, p. 326). For the crystalline form of pure leucin obtained as a decomposition product of proteid see Fig. 24, p. 69. Tyrosin crystallizes in urinary sediments in the well known sheaf or tuft formation (Fig. 23, p. 68). For other tests on leucin and tyrosin see pages 80 to 82.

FIG. 104.

CRYSTALS OF IMPURE LEUCIN.
(Ogden.)

Hæmatoidin and Bilirubin.—There are divergent opinions regarding the occurrence of these bodies in urinary sediment. Each of them crystallizes in the form of tufts of small needles or in the form of small plates which are ordinarily yellowish-red in color (Fig. 41, p. 119). Because of the fact that the crystalline form of the two substances is identical many investigators claim them to be one and the same body. Other investigators claim, that while the crystalline form is the same in each case, that there are certain chem-

ical differences which may be brought out very strikingly by properly testing. For instance, it has been claimed that hæmatoidin may be differentiated from bilirubin through the fact that it gives a momentary color reaction (blue) when nitric acid is brought in contact with it, and further, that it is not dissolved on treatment with ether or potassium hydroxide. Pathologically, typical crystals of hæmatoidin or bilirubin have been found in the urinary sediment in jaundice, acute yellow atrophy of the liver, carcinoma of the liver, cirrhosis of the liver, and in phosphorus poisoning, typhoid fever and scarlatina.

Magnesium Phosphate.—Magnesium phosphate crystals occur rather infrequently in the sediment of urine which is neutral, alkaline or *feebly* acid in reaction. It ordinarily crystallizes in elongated, highly refractive, rhombic plates which are soluble in acetic acid.

Indigo.—Indigo crystals are frequently found in urine which has undergone alkaline fermentation. They result from the breaking down of indoxyl-sulphates or indoxyl-glycuronates. Ordinarily indigo deposits as dark blue stellate needles or occurs as amorphous particles or broken fragments. These crystalline or amorphous forms may occur in the sediment or may form a blue film on the surface of the urine. Indigo crystals generally occur in urine which is alkaline in reaction, but they have been detected in acid urine.

Xanthin.—Xanthin is a constituent of normal urine but is found in the sediment in crystalline form very infrequently, and then only in pathological urine. When present in the sediment xanthin generally occurs in the form of whetstone-shaped crystals somewhat similar in form to the whetstone variety of uric acid crystal. They may be differentiated from uric acid by the great ease with which they may be brought into solution in dilute ammonia and on applying heat. Xanthin may also form urinary calculi. The clinical significance of xanthin in urinary sediment is not well understood.

Melanin.—Melanin is an extremely rare constituent of

urinary sediments. Ordinarily in melanuria the melanin remains in solution; if it separates it is generally held in suspension as fine amorphous granules.

(b) Organized Sediments.

Epithelial cells.

Pus cells.

Casts { Hyaline.
Granular.
Epithelial.
Blood.
Fatty.
Waxy.
Pus.

Cylindroids.

Erythrocytes.

Spermatozoa.

Urethral filaments.

Tissue debris.

Animal parasites.

Micro-organisms.

Fibrin.

Foreign substances due to contamination.

Epithelial Cells.—The detection of a certain number of these cells in urinary sediment is not, of itself, a pathological sign, since they occur in normal urine. However, in certain pathological conditions they are greatly *increased* in number, and since different areas of the urinary tract are lined with different forms of epithelial cells, it becomes necessary, when examining urinary sediments, to note not only the relative number of such cells, but at the same time to carefully observe the shape of the various individuals in order to determine, as far as possible, from what portion of the tract they have been derived. Since the different layers of the epithelial lining are composed of cells different in form from those of the associated layers, it is evident that a careful microscopical examination of these cells may tell us the par-

ticular layer which is being desquamated. It is frequently a most difficult undertaking, however, to make a clear differentiation between the various forms of epithelial cells present in a sediment. If skilfully done, such a microscopical differentiation may prove to be of very great diagnostic aid.

The principal forms of epithelial cells met with in urinary sediments are shown in Fig. 105, below.

FIG. 105.



EPITHELIUM FROM DIFFERENT AREAS OF THE URINARY TRACT.

a, Leucocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, *g*, *g*, *g*, squamous epithelium from the bladder; *h*, *h*, neck-of-bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, *l*, scaly epithelium; *m*, *m'*, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell. (Ogden.)

Pus Cells.—Pus corpuscles or leucocytes are present in extremely small numbers in normal urine. Any considerable increase in the number, however, ordinarily denotes a pathological condition, generally an acute or chronic inflammatory condition of some portion of the urinary tract. The sudden appearance of a large amount of pus in a sediment denotes the opening of an abscess into the urinary tract. Other form elements, such as epithelial cells, casts, etc., ordinarily accom-

pany pus corpuscles in urinary sediment and a careful examination of these associated elements is necessary in order to form a correct diagnosis as to the origin of the pus. Proteid is always present in urine which contains pus.

FIG. 106.

PUS CORPUSCLES. (After *Ultrmann*.)

1, Normal; 2, showing amoeboid movements; 3, nuclei rendered distinct by acetic acid; 4, as observed in chronic pyelitis; 5, swollen by ammonium carbonate.

The appearance which pus corpuscles exhibit under the microscope depends greatly upon the reaction of the urine containing them. In acid urine they generally present the appearance of round, colorless cells composed of refractive, granular protoplasm, and may frequently exhibit amoeboid movements, especially if the slide containing them be warmed slightly. They are nucleated (one or more nuclei), the nuclei being clearly visible only upon treating the cells with water, acetic acid or some other suitable reagent. In urine which has a decided alkaline reaction, on the other hand, the pus corpuscles are often greatly degenerated. They may be seen as swollen, transparent cells, which exhibit no granular structure and as the process of degeneration continues the cell outline ceases to be visible, the nuclei fade, and finally only a mass

of debris containing isolated nuclei and an occasional cell remains.

It is frequently rather difficult to make a differentiation between pus corpuscles and certain types of epithelial cells which are similar in form. Such confusion may be avoided by the addition of iodine solution (I in KI), a reagent which stains the pus corpuscles a deep mahogany-brown and transmits to the epithelial cells a light yellow tint. The test proposed by Vitali often gives very satisfactory results. This simply consists in acidifying the urine (if alkaline) with acetic acid, then filtering, and treating the sediment on the filter paper with freshly prepared tincture of guaiac. The presence of pus in the sediment is indicated if a *blue* color is observed. Large numbers of pus corpuscles are present in the urinary sediment in gonorrhœa, leucorrhœa, chronic pyelitis and in abscess of the kidney.

FIG. 107.



HYALINE CASTS.

One cast is impregnated with four renal cells.

Casts.—These are cylindrical formations, which originate in the uriniferous tubules and are forced out by the pressure of the urine. They vary greatly in size but in nearly every instance they possess parallel sides and rounded ends. The finding of casts in the urine is very important because of the fact that they *generally indicate* some kidney disorder; if albumin accompanies the casts the indication is much accentuated. Casts have been classified according to their microscopical characteristics as follows: (a) Hyaline, (b) granular, (c) epithelial, (d) blood, (e) fatty, (f) waxy, (g) pus.

(a) *Hyaline Casts.*—These are composed of a basic material which is transparent, homogeneous and very light in color (Fig. 107, p. 331). In fact, chiefly because of these physical

FIG. 108.



GRANULAR CASTS. (After Peyer.)

properties, they are the most difficult form of renal cast to detect under the microscope. Frequently such casts are impregnated with deposits of various forms such as erythrocytes, epithelial cells, fat globules, etc., thus rendering the form of

the cast more plainly visible. Staining is often resorted to in order to render the shape and character of the cast more easily determined. Ordinary iodine solution (I in KI) may be used in this connection; many of the anilin dyes are also in common use for this purpose, *e. g.*, gentian-violet, Bismarck-brown, methylene-blue, fuchsin and eosin. Generally, but not always, albumin is present in urine containing hyaline casts. Hyaline casts are common to all kidney disorders, but occur particularly in the earliest and recovering stages of parenchymatous nephritis and in interstitial nephritis.

(b) *Granular Casts.*—The common hyaline material is ordinarily the basic substance of this form of cast. The granular material generally consists of albumin, epithelial cells, fat or

FIG. 109.



GRANULAR CASTS.

a, Finely granular; *b*, coarsely granular.

FIG. 110.



EPITHELIAL CASTS.

disintegrated erythrocytes or leucocytes, the character of the cast varying according to the nature and size of the granules (Fig. 108, page 332, and Fig. 109, above). Thus we have casts of this general type classified as *finely granular* and *coarsely granular* casts. Granular casts, and in particular the finely granular types, occur in the sediment in practically every kidney disorder but are probably especially characteristic of the sediment in inflammatory disorders.

(c) *Epithelial Casts*.—These are casts bearing upon their surface epithelial cells from the lining of the uriniferous tubules (Fig. 110, p. 333). The basic material of this form of cast may be hyaline or granular in nature. Epithelial casts are particularly abundant in the urinary sediment in acute nephritis.

(d) *Blood Casts*.—Casts of this type may consist of erythrocytes borne upon a hyaline or a fibrous basis (Fig. 111, below). The occurrence of such casts in the urinary sediment

FIG. 111.



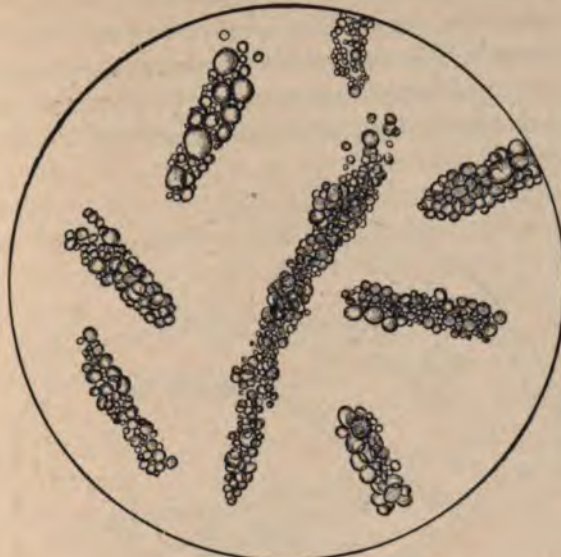
BLOOD, PUS, HYALINE AND EPITHELIAL CASTS.

a, Blood casts; b, pus cast; c, hyaline cast impregnated with renal cells; d, epithelial casts.

denotes renal hæmorrhage and they are considered to be especially characteristic of acute diffuse nephritis and acute congestion of the kidney.

(e) *Fatty Casts*.—Fatty casts may be formed by the deposition of fat globules or crystals of fatty acid upon the surface of a hyaline or granular cast (Fig. 112). In order to

FIG. 112.



FATTY CASTS. (After Peyer.)

FIG. 113.



a, Fatty casts; o, FATTY AND WA

constitute a true fatty cast the deposited material must cover the greater part of the surface area of the cast. The presence of fatty casts in urinary sediment indicates fatty degeneration of the kidney; such casts are particularly characteristic of sub-acute and chronic inflammations of the kidney.

FIG. 114.



CYLINDROIDS. (After Peyer.)

(f) *Waxy Casts*.—These casts possess a basic substance similar to that which enters into the foundation of the hyaline form of cast. In common with the hyaline type they are colorless, refractive bodies but differ from this form of cast in being, in general, of greater length and diameter and possessing sharper outlines and a light yellow color (Fig. 113, p. 335). Such casts occur in several forms of nephritis but do not appear to characterize any particular type of the disorder except *amyloid disease*, in which they are rather common.

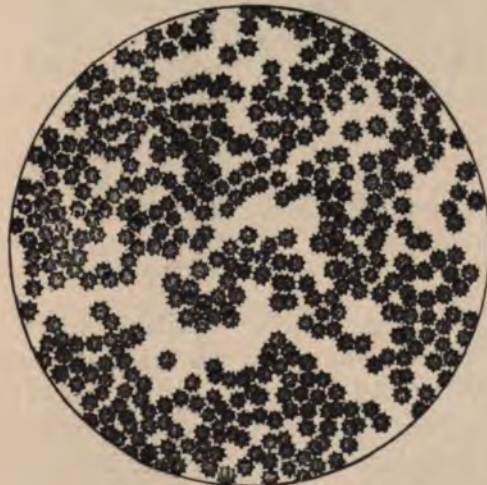
(g) *Pus Casts*.—Casts whose surface is covered with pus cells or leucocytes are termed *pus casts* (Fig. 111, p. 334). They are frequently mistaken for epithelial casts. The differentia-

tion between these two types is made very simple however by treating the cast with acetic acid which causes the nuclei of the leucocytes to become plainly visible. The true pus cast is quite rare and indicates renal suppuration.

Cylindroids.—These formations may occur in normal or pathological urine and have no particular clinical significance. They are frequently mistaken for true casts, especially the hyaline type, but they are ordinarily *flat* in structure with a rather smaller diameter than casts, may possess forked or branching ends and are not composed of homogeneous material as are the hyaline casts. Such "false casts" may become coated with urates, in which event they appear granular in structure. The basic substance of cylindroids is often the nucleo-proteid of the urine (see Fig. 114, page 336).

Erythrocytes.—These form elements are present in the urinary sediment in various diseases. They may appear as

FIG. 115.



CRENATED ERYTHROCYTES.

the normal biconcave, yellow erythrocyte (Plate IV, opposite page 151) or may exhibit certain modifications in form such as the crenated type (Fig. 115, above) which is often seen in con-

centrated urine. Under different conditions they may become swollen sufficiently to entirely erase the biconcave appearance and may even occur in the form of colorless spheres having a smaller diameter than the original disc-shaped corpuscles. Erythrocytes are found in urinary sediment in hemorrhage of the kidney or of the urinary tract, in traumatic hemorrhage, hemorrhage from congestion and in hemorrhagic diathesis.

Spermatozoa.—Spermatozoa may be detected in the urinary sediment in diseases of the genital organs, as well as after coitus, nocturnal emissions, epileptic and other convulsive

FIG. 116.



HUMAN SPERMATOZOA.

attacks and sometimes in severe febrile disorders, especially in typhoid fever. In form they consist of an oval body, to which is attached a long, delicate tail (Fig. 116, above). Upon examination they may show motility or may be motionless.

Urethral Filaments.—These are peculiar thread-like bodies which are sometimes found in urinary sediment. They may occasionally be detected in normal urine and pathologically are found in the sediment in acute and chronic gonorrhœa and in urethrorrhœa. The ground-substance of these urethral filaments is in part, at least, similar to that of the cylindroids (see

page 337). The urine first voided in the morning is best adapted for the examination for filaments. These filaments may ordinarily be removed by a pipette since they are generally macroscopic.

Tissue Debris.—Masses of cells or fragments of tissue are frequently found in urinary sediment. They may be found in the sediment in tubercular affections of the kidney and urinary tract or in tumors of these organs. Ordinarily it is necessary to make a histological examination of such tissue fragments before coming to a final decision as to their origin.

Animal Parasites.—The cysts, hooklets and membrane shreds of *echinococci* are sometimes found in urinary sediments. Other animal organisms which are more rarely met with in the urine are embryos of the *Filaria sanguinis* and eggs of the *Distoma hæmatobium* and *Ascarides*. Animal parasites in general occur most frequently in the urine in tropical countries.

Micro-Organisms.—Bacteria as well as yeast and moulds are frequently detected in the urine. Both the pathogenic and non-pathogenic forms of bacteria may occur. The non-pathogenic forms most frequently observed are *micrococcus ureæ*, *bacillus ureæ*, and *staphylococcus ureæ liquefaciens*. Of the pathogenic forms many have been observed, *e. g.*, *Bacterium Coli*, *typhoid bacillus*, *tubercle bacillus*, *gonococcus*, *bacillus pyocyaneus* and *proteus vulgaris*. Yeast and moulds are most frequently met with in diabetic urine.

Fibrin.—Following hæmaturia, fibrin clots are occasionally observed in the urinary sediment. They are generally of a semi-gelatinous consistency and of a very light color, and when examined under the microscope they are seen to be composed of bundles of highly refractive fibres which run parallel.

Foreign Substances Due to Contamination.—Such foreign substances as fibers of silk, linen or wool; starch granules, hair, fat and sputum, as well as muscle fibers, vegetable cells and food particles are often found in the urine. Care should be taken that these foreign substances are not mistaken for any of the true sedimentary constituents already mentioned.

CHAPTER XX.

URINE: CALCULI.

Urinary *calculi*, also called *concretions*, or *concrements* are solid masses of urinary sediment formed in some part of the urinary tract. They vary in shape and size according to their location, the smaller calculi termed *sand* or *gravel* in general arising from the kidney or the pelvic portion of the kidney, whereas the large calculi are ordinarily formed in the bladder. There are two general classes of calculi as regards composition, *i. e.*, *simple* and *compound*. The simple form is made up of but a single constituent whereas the compound type contains two or more individual constituents. The structural plan of most calculi consists of an arrangement of concentric rings about a central nucleus, the number of rings frequently being dependent upon the number of individual constituents which enter into the structure of the calculus. In case two or more calculi unite to form a single calculus the resultant body will obviously contain as many nuclei as there were individual calculi concerned in its construction. Under certain conditions the growth of a calculus will be principally in only one direction thus preventing the nucleus from maintaining a central location. The qualitative composition of urinary calculi is dependent, in great part, upon the reaction of the urine *e. g.*, if the reaction of the urine is acid the calculi present will be composed, in great part at least, of substances that are capable of depositing in acid urine.

According to Ultzmann, out of 545 cases of urinary calculus, uric acid and urates formed the nucleus in about 81 per cent of the cases; earthy phosphates in about 9 per cent; calcium oxalate in about 6 per cent; cystin in something over 1 per cent, while in about 3 per cent of the cases some foreign body comprised the nucleus.

In the chemical examination of urinary calculi the most valuable data are obtained by subjecting each of the concentric layers of the calculus to a separate analysis. Material for examination may be conveniently obtained by sawing the calculus carefully through the nucleus, then separating the various layers or by scraping off from each layer (without separating the layers) enough powder to conduct the examination as outlined in the scheme (see page 343).

Varieties of Calculus.

Uric Acid and Urate Calculi.—Uric acid and urates constitute the nuclei of a large proportion (81 per cent) of urinary concretions. Such stones are always colored, the tint varying from a pale yellow to a brownish-red. The surface of such calculi is generally smooth but it may be rough and uneven.

Phosphatic Calculi.—Ordinarily these concretions consist principally of "triple phosphate" and other phosphates of the alkaline earths, with very frequent admixtures of urates and oxalates. The surface of such calculi is generally rough but may occasionally be rather smooth. The calculi are somewhat variable in color exhibiting gray, white or yellow tints under different conditions. When composed of earthy phosphates the calculi are characterized by their friability.

Calcium Oxalate Calculi.—This is the hardest form of calculus to deal with, and is rather difficult to crush. They ordinarily occur in two general forms, *i. e.*, the small, smooth concretion which is characterized as the *hemp-seed calculus* and the medium-sized or large stone possessing an extremely uneven surface which is generally classed as a *mulberry calculus*. This roughened surface of the latter form of calculus is due, in many instances, to protruding calcium oxalate crystals of the octahedral type.

Calcium Carbonate Calculi.—Calcium carbonate concretions are quite common in herbivorous animals but of exceedingly rare occurrence in man. They are generally small, white or grayish calculi, spherical in form and possess a hard, smooth surface.

Cystin Calculi.—The cystin calculus is a rare variety of calculus. Ordinarily they occur as small, smooth, oval or cylindrical concretions which are white or yellow in color and of a rather soft consistency.

Xanthin Calculi.—This form of calculus is somewhat more rare than the cystin type. The color may vary from white to brownish-yellow. Very often uric acid and urates are associated with xanthin in this type of calculus. Upon rubbing a xanthin calculus it has the property of assuming a wax-like appearance.

Urostealith Calculi.—This form of calculus is extremely rare. Such concretions are composed principally of fat and fatty acid. When moist they are soft and elastic but when dried they become brittle. Urostealiths are generally light in color.

Fibrin Calculi.—Fibrin calculi are produced in the process of blood coagulation within the urinary tract. They frequently occur as nuclei of other forms of calculus. They are rarely found.

Cholesterin Calculi.—An extremely rare form of calculus somewhat resembling the cystin type.

Indigo Calculi.—Indigo calculi are extremely rare, only two cases having been reported. One of these indigo calculi is on exhibition in the museum of Jefferson Medical College of Philadelphia.

The scheme, proposed by Heller and given on page 343, will be found of much assistance in the chemical examination of urinary calculi.

Does not burn

The powder when treated with HCl

Does not effervesce

The gently-heated powder with HCl

The powder when moistened
with a little KOH

Abundant ammonia. The powder of crystalline precipitate with ammonia

Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives

“Triple phosphate” (mixed with unknown amount of early phosphate).

No ammonia or, at least, only traces of ammonia. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous)

Bone-earth (magnesium and calcium phosphate).

Effervesces

Effervesces

Calcium carbonate.

Calcium oxalate.

With flame

Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia.

Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether.

Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in KOH with heat. Precipitated herefrom by acetic acid and generation of H_2S .

Urostealith.

Fibrin.

	Without flame
1	100
2	100
3	100
4	100
5	100
6	100
7	100
8	100
9	100
10	100
11	100
12	100
13	100
14	100
15	100
16	100
17	100
18	100
19	100
20	100
21	100
22	100
23	100
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78	100
79	100
80	100
81	100
82	100
83	100
84	100
85	100
86	100
87	100
88	100
89	100
90	100
91	100
92	100
93	100
94	100
95	100
96	100
97	100
98	100
99	100
100	100

The powder gives the murexid test

The powder when treated with KOH gives

No noticeable ammonia reaction

Strong ammonia reaction

Uric acid.

Ammonium urate.

CHAPTER XXI.

URINE: QUANTITATIVE ANALYSIS.

I. Proteid.

1. **Scherer's Coagulation Method.**—The content of *coagulable* proteid may be accurately determined as follows: Place 50 c.c. of urine in a small beaker and raise the temperature of the fluid to about 40°C . upon a water-bath. Add dilute acetic acid, drop by drop, to the warm urine, to precipitate the proteid which will separate in a flocculent form. Care should be taken not to add too much acid; ordinarily less than twenty drops is sufficient. The temperature of the water in the water-bath should now be raised to the boiling-point and maintained there for a few minutes in order to insure the complete coagulation of the proteid present. Now filter the urine through a previously *washed, dried and weighed* filter paper, wash the precipitated proteid, in turn, with hot water, 95 per cent alcohol and with ether, and dry the paper and precipitate, to constant weight, in an air-bath at 110°C . Subtract the weight of the filter paper from the combined weight of the paper and precipitate and calculate the percentage of proteid in the urine specimen.

Calculation.—To determine the percentage of proteid present in the urine under examination, multiply the weight of the precipitate, expressed in grams, by 2.

2. **Esbach's Method.**—This method depends upon the precipitation of proteid by Esbach's reagent¹ and the apparatus used in the estimation is Esbach's albuminometer (Fig. 117, p. 345). In making a determination fill the albuminometer to the point U with urine, then introduce the reagent until the point R is reached. Now stopper the tube, invert it slowly

¹ Esbach's reagent is prepared by dissolving 10 grams of picric acid and 20 grams of citric acid in 1 liter of water.

several times in order to insure the thorough mixing of the fluids and stand the tube aside for 24 hours. Creatinin, resin acids, etc., are precipitated in this method, and for this and other reasons it is not as accurate as the coagulation method. It is, however, extensively used clinically.

Calculation.—The graduations on the albuminometer indicate *grams of proteid per liter of urine*. Thus, if the proteid precipitate is level with the figure 3 of the graduated scale this denotes that the urine examined contains 3 grams of proteid to the liter. To express the amount of proteid in *per cent* simply move the decimal point *one* place to the left. In the case under consideration the urine contains 0.3 per cent of proteid.

II. Dextrose.

1. **Fehling's Method.**—Place 10 c.c. of the urine under examination in a 100 c.c. volumetric flask and make the volume up to 100 c.c. with distilled water. Thoroughly mix this diluted urine, by pouring it into a beaker and stirring with a glass rod, then transfer a portion of it to a burette which is properly supported in a clamp.

Now place 10 c.c. of Fehling's solution¹ in a small beaker, dilute it with approximately 40 c.c. of distilled water, heat to boiling, and observe whether decomposition of the Fehling's solution itself has occurred as indicated by the production of a turbidity. If such turbidity is produced the Fehling's solution is unfit for use. Clamp the burette containing the diluted urine immediately over the beaker and carefully allow from 0.5

¹ Directions for the preparation of Fehling's solution are given in a note at the bottom of page 8.

FIG. 117.



ESBACH'S ALBUMINOMETER. (Ogden.)

to 1 c.c. of the diluted urine to flow into the *boiling* Fehling's solution. Bring the solution to the boiling-point after each addition of urine and continue running in the urine from the burette, 0.5-1 c.c. at a time, as indicated, until the Fehling's solution is *completely reduced*, i. e., until all the cupric oxide in solution has been precipitated as cuprous oxide. This point will be indicated by the *absolute disappearance of all blue color*. When this end-point is reached note the number of cubic centimeters of diluted urine used in the process and calculate the percentage of dextrose present, in the sample of urine analyzed, according to the method given on page 347.

This is a very satisfactory method, the main objection to its use being the uncertainty attending the determination of the end-reaction, i. e., the difficulty with which the exact point where the blue color *finally disappears* is noted. Several means of accurately fixing this point have been suggested but they are practically all open to objection. As good a "check" as any, perhaps, is to filter a few drops of the solution, through a double paper, after the blue color has *apparently* disappeared, acidify the filtrate with acetic acid and add potassium ferrocyanide. If the copper of the Fehling's solution has been completely reduced, there will be no color reaction, whereas the production of a brown color indicates the presence of *unreduced* copper. Harrison has recently suggested the following procedure to determine the exact end-point: To about 1 c.c. of a starch iodide solution¹ in a test-tube add 2-3 drops of acetic acid and introduce into the acidified mixture 1-2 drops of the solution to be tested. *Unreduced copper* will be indicated by the production of a *purplish-red* or *blue* color due to the liberation of iodine.

It is ordinarily customary to make at least three deter-

¹ The starch-iodide solution may be prepared as follows: Mix 0.1 gram of starch with *cold* water in a mortar and pour the suspended starch granules into 75-100 c.c. of *boiling* water, stirring continuously. Cool the starch paste, add 20-25 grams of potassium iodide and dilute the mixture to 250 c.c. This solution deteriorates upon standing, and therefore must be freshly prepared as needed.

minations by Fehling's method before coming to a final conclusion regarding the sugar content of the urine under examination.

Calculation.—Ten c.c. of Fehling's solution is completely reduced by 0.05 gram of *dextrose*. If y represents the number of cubic centimeters of *undiluted* urine (obtained by dividing the burette reading by 10) necessary to reduce the 10 c.c. of Fehling's solution, we have the following proportion:

$$y:0.05::100:x \text{ (percentage of dextrose).}$$

2. **Purdy's Method.**—Purdy's solution¹ is a modification of Fehling's solution and is said to possess greater stability than the latter. One of the most satisfactory points about the method as suggested by Purdy is the ease with which the exact end-reaction may be determined. In determining the percentage of dextrose by this method proceed as follows: Place 35 c.c. of Purdy's solution in a 200 c.c. Erlenmeyer flask and dilute the fluid with approximately two volumes of distilled water. Fit a cork, provided with two perforations, to the neck of the flask and through one perforation introduce the tip of a burette and through the second perforation introduce a tube bent at right angles in such a manner as to allow the steam to escape and keep the fumes of ammonia away from the face of the operator as completely as possible.¹ Now

¹ Purdy's solution has the following composition:

Cupric sulphate	4.752 grams.
Potassium hydroxide	23.5 grams.
Ammonia (U. S. P., sp. gr. 0.9).....	350.0 c.c.
Glycerin	38.0 c.c.
Distilled water, to make total volume 1 liter.	

In preparing the solution bring the CuSO_4 and KOH into solution in separate vessels, mix the two solutions, cool the mixture and add the ammonia and glycerin. After this has been done the total volume should be made up to 1 liter with distilled water.

Thirty-five cubic centimeters of Purdy's solution is exactly reduced by 0.02 gram of dextrose.

¹This side tube may also be equipped with a simple air-valve, thus insuring the exclusion of air and thereby contributing to the accuracy of

bring the solution to the boiling-point and add the urine, drop by drop, until the intensity of the *blue color begins to diminish*. When this point is reached add the urine somewhat *more slowly* until the blue color is entirely dissipated and an *absolutely decolorized* solution remains. Take the burette reading and calculate the percentage of dextrose in the urine examined according to the method given below.

Care should be taken not to boil the solution for too long a period, since, under these conditions, sufficient ammonia might be lost to allow the cuprous hydroxide, CuOH , to precipitate.

Some investigators consider it to be advisable to dilute the urine before applying the above manipulation, but ordinarily this is not necessary unless the urine has a high content of dextrose (5 per cent or over). In this event the urine may be diluted with 2-3 volumes of water and the proper correction made in the calculation.

Calculation.—Thirty-five c.c. of Purdy's solution is completely reduced by 0.02 gram of dextrose. If y represents the number of cubic centimeters of *undiluted* urine necessary to reduce 35 c.c. of Purdy's solution, we have the following proportion:

$$y : 0.02 :: 100 : x \text{ (percentage of dextrose).}$$

3. Fermentation Method.—This method consists in the measurement of the volume of CO_2 evolved when the dextrose of the urine undergoes fermentation with yeast. None of the various methods whose manipulation is based upon this principle is *absolutely* accurate. The method in which Einhorn's saccharometer (Fig. 2, page 10) is the apparatus employed is perhaps as satisfactory as any for clinical purposes. The procedure is as follows: Place about 15 c.c. of urine in the determination, inasmuch as the cuprous salts would be reoxidized upon coming in contact with the air. If one is careful to maintain the solution continuously at the boiling-point throughout the entire process, however, there is no opportunity for air to enter and therefore no need of an air-valve.

a mortar, add about 1 gram of yeast ($\frac{1}{16}$ of the ordinary cake of compressed yeast) and carefully crush the latter by means of a pestle. Transfer the mixture to the saccharometer, being careful to note that the graduated tube is *completely* filled and that no air bubbles gather at the top. Allow the apparatus to stand in a warm place (30°C.) for 12 hours and observe the percentage of dextrose as indicated by the graduated scale of the instrument. Both the percentage of dextrose and the number of cubic centimeters of CO_2 are indicated by the graduations on the side of the saccharometer tube.

4. **Polariscopic Examination.**—Before subjecting urine to a polariscopic examination the slightly acid fluid should be decolorized as thoroughly as possible by the addition of a little plumbic acetate. The urine should be well stirred and then filtered through a filter paper which has not been previously moistened. In this way a perfectly *clear* and almost colorless liquid is obtained.

In determining dextrose by means of the polariscope it should be borne in mind that this carbohydrate is often accompanied by other optically active substances, such as proteids, lævulose, β -oxybutyric acid and conjugate glycuronates which may introduce an error into the polariscopic reading; the method is, however, sufficiently accurate for practical purposes.

For directions as to the manipulation of the polariscope see page 11.

III. Uric Acid.

1. **Folin-Shaffer Method.**—Introduce 100 c.c.¹ of urine into a beaker, add 25 c.c. of the Folin-Shaffer reagent² and allow the mixture to stand,³ without further stirring, until the precipitate has settled (5–10 minutes). Filter, transfer

¹It is preferable to use more than 100 c.c. of urine if the fluid has a specific gravity less than 1.020.

²The Folin-Shaffer reagent consists of 500 grams of ammonium sulphate, 5 grams of uranium acetate and 60 c.c. of 10 per cent acetic acid in 650 c.c. of distilled water.

³The mixture should not be allowed to stand for too long a time at this point, since uric acid may be lost through precipitation.

100 c.c. of the filtrate to a beaker, add 5 c.c. of concentrated ammonia and allow the mixture to stand for 24 hours. Transfer the precipitated ammonium urate quantitatively to a filter plant,¹ using 10 per cent ammonium sulphate to remove the final traces of the urate from the beaker. Wash the precipitate *approximately* free from chlorides by means of 10 per cent ammonium sulphate solution, remove the paper from the funnel, open it and by means of *hot* water rinse the precipitate back into the beaker in which the urate was originally precipitated. The volume of fluid at this point should be about 100 c.c. Cool the solution to room temperature, add 15 c.c. of concentrated sulphuric acid and titrate at once with $\frac{N}{20}$ potassium permanganate, $K_2Mn_2O_8$, solution. The first tinge of pink color which extends throughout the fluid after the addition of *two drops* of the permanganate solution, while stirring with a glass rod, should be taken as the end-reaction. Take the burette reading and compute the percentage of uric acid present in the urine under examination.

Calculation.—Each cubic centimeter of $\frac{N}{20}$ potassium permanganate solution is equivalent to 3.75 milligrams (0.00375 gram) of uric acid. The 100 c.c. from which the ammonium urate was precipitated is equivalent to only four-fifths of the 100 c.c. of urine originally taken, therefore we must take five-fourths of the burette reading in order to ascertain the number of cubic centimeters of the permanganate solution required to titrate 100 c.c. of the *original urine* to the correct end-point. If y represents the number of cubic centimeters of the permanganate solution required, we may make the following calculation:

$$y \times 0.00375 = \text{weight of uric acid in 100 c.c. of urine.}$$

Calculate the quantity of uric acid in the twenty-four hour urine specimen.

2. Heintz Method.—This is a very simple method and was the first one in general use for the quantitative determi-

¹ The Schleicher and Schüll *hardened* papers are the best for this purpose.

nation of uric acid. It is believed to be somewhat less accurate than the method just described. The procedure is as follows: Place 100 c.c. of filtered urine in a beaker, add 5 c.c. of concentrated hydrochloric acid, stir the fluid thoroughly and stand it away in a cool place for 24 hours. Filter off the uric acid crystals upon a washed, dried and *weighed* filter paper and wash them with *cold* distilled water, a few cubic centimeters at a time until the chlorides are removed. Now wash, in turn, with alcohol and with ether and finally dry the paper and crystals to constant weight at 110° C. In the process of washing the uric acid free from chlorides an error is introduced, since every cubic centimeter of water so used dissolves 0.00004 gram of uric acid. For this reason a correction is necessary. It has been suggested that the pigment of the crystals is equivalent in weight to the amount of uric acid dissolved by the first 30 c.c. of water, and this factor should be taken into account in the computation of the percentage of uric acid.

Calculation.—Since 100 c.c. of urine was used the *corrected* weight of the uric acid crystals, in grams, will express the *percentage* of uric acid present.

IV. Urea.

1. Knop-Hüfner Hypobromite Method (using Marshall's Urea Apparatus).—Place the thumb over the side opening of the bulbed-tube of the apparatus (Fig. 118, p. 352) and carefully fill the tube with sodium hypobromite solution.¹ Close the opening in the end of the tube with a rubber stopper, incline the tube to allow air-bubbles to escape

¹ The ingredients of the sodium hypobromite solution should be prepared in the form of *two* separate solutions. When needed for use mix equal volumes of solution *a*, solution *b* and water.

(*a*) Dissolve 125 grams of sodium bromide in water, add 125 grams of bromine and make the total volume of the solution 1 liter.

(*b*) A solution of sodium hydroxide having a specific gravity of 1.250. This is approximately a 22.5 per cent solution.

Preserve both solutions in rubber-stoppered bottles.

and finally invert the tube and fix the stoppered end in the saucer-shaped vessel. By means of the graduated pipette rapidly introduce 1 c.c. of urine¹ into the hypobromite

FIG. 118.

MARSHALL'S UREA APPARATUS.
(Tyson.)

a, Bulbed measuring tube; b, saucer-shaped vessel; c, graduated pipette; d, funnel-tube.

solution through the side opening of the bulbed-tube. Withdraw the pipette immediately after the urine has been introduced. When the decomposition of the urea is completed (10-20 minutes) gently tap the bulbed-tube with the finger in order to dislodge any gas-bubbles which may have collected on the inner surface of the glass. The atmospheric pressure should now be equalized by attaching the funnel-tube to the bulbed-tube at the side opening and introducing hypobromite solution into it until the columns of liquid in the two tubes are uniform in height. The graduated scale of the bulbed-tube should now be read in order to determine the number of

cubic centimeters of nitrogen gas evolved. By means of the appended formula the *weight* of the urea present in the urine under examination may be computed.

*Calculation.*²—By properly substituting in the following formula the *weight* of urea, in grams, contained in the volume of urine decomposed (1 c.c. or more) may readily be determined:

¹ Ordinarily 1 c.c. of urine is sufficient; more may be used, however, if its content of urea is *very low*.

² 0.003665 = coefficient of expansion of gases for 1° C. 354.5 = number of c.c. of nitrogen gas evolved from 1 gram of urea.

$$w = \frac{v(p - T)}{354.5 + 760(1 + 0.003665t)}$$

w = weight of urea, in grams.

v = observed volume of nitrogen expressed in cubic centimeters.

p = barometric pressure expressed in mm. of mercury.

T = tension of aqueous vapor¹ for temperature t .

t = temperature (centigrade).

If we wish to calculate the *percentage* of urea we may do so by means of the following proportion in which y represents the volume of urine used and w denotes the weight of the urea contained in the volume y :

$$y:w::100:x \text{ (percentage of urea).}$$

Sodium hypobromite solution may also be employed for the determination of urea in the apparatus devised by Hüfner which is pictured in Fig. 119, page 354.

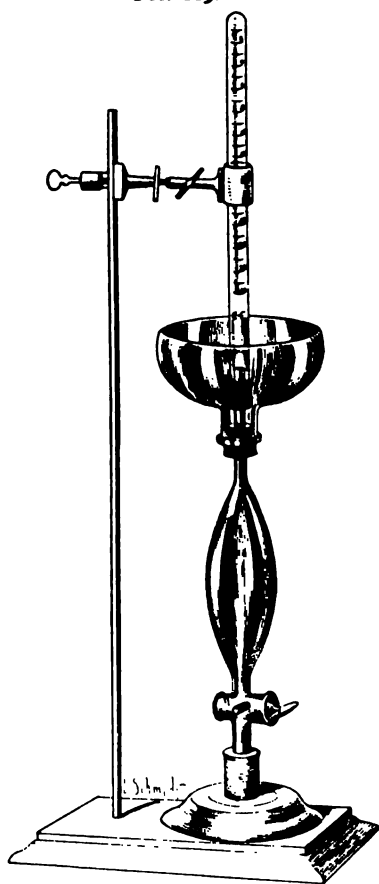
2. Knop-Hüfner Hypobromite Method (using the Doremus-Hinds Ureometer).—In common with the method already described this method depends upon the measurement of the volume of nitrogen gas liberated when the urea of the urine is decomposed by means of sodium hypobromite solution. The Doremus-Hinds ureometer (Fig. 120, p. 355), is one of the simplest and cheapest forms of apparatus in general use for the determination of urea by the hypobromite process. In using this apparatus proceed as follows: Fill the side tube B and the lumen of the stopcock C with the urine

¹The values of T for the temperatures ordinarily met with are given in the following table:

Temp.	Tension in mm.	Temp.	Tension in mm.
15° C.....	12.677	21° C.....	18.505
16° C.....	13.519	22° C.....	19.675
17° C.....	14.009	23° C.....	20.909
18° C.....	15.351	24° C.....	22.211
19° C.....	16.345	25° C.....	23.582
20° C.....	17.396		

under examination. Carefully wash out tube A with water and introduce into it sodium hypobromite solution¹ being careful to fill the bulb sufficiently full to prevent the entrance of air

FIG. 119.



HÜLNER'S UREA APPARATUS.

into the graduated portion. Now allow 1 c.c. of urine to flow from tube B into tube A and after the evolution of gas bubbles has ceased (10–20 minutes) take the reading of the graduated scale on tube A.

In common with all other methods which are based upon the decomposition of urea by means of hypobromite solution, this method is not absolutely correct. It is, however, sufficiently accurate for ordinary clinical purposes.

Calculation.—Observe the reading on the graduated scale of tube A. This tube is so graduated as to represent the weight of urea in grams, per cubic centimeter of urine. If we wish to compute the *percentage* of urea present this may be done very readily by simply moving the decimal point *two*

places to the right, e. g., if the reading is 0.02 gram the urine contains 2 per cent of urea.

¹For directions as to the preparation of this solution see page 351.

²If the content of urea in the urine under examination is *large*, the urine may be diluted with water before determining the urea. If this is done it must of course be taken into consideration in computing the content of urea.

3. **Folin's Method.**—This is one of the most accurate methods yet devised for the determination of urea in the urine. The procedure is as follows: Place 5 c.c. of urine in a 200 c.c. Erlenmeyer flask and add to it 5 c.c. of concentrated hydrochloric acid, 20 grams of crystallized magnesium chloride, a piece of paraffin the size of a hazel nut and 2-3 drops of a 1 per cent aqueous solution of "alizarin red." Insert a Folin safety tube (Fig. 121, p. 356) into the neck of the flask and boil the mixture until each drop of reflow from the safety tube produces a very perceptible bump; the heat is then reduced somewhat and continued one hour.¹ The contents of the flask must not remain alkaline and to obviate this, at the first appearance of a reddish tinge in the contents of the flask *a few drops* of the acid distillate are shaken back into the flask. At the end of an hour the contents of the vessel are transferred to a 1 liter flask with about 700 c.c. of distilled water, about 20 c.c. of 10 per cent potassium hydroxide or sodium hydroxide solution is added and the mixture distilled into a known volume of $\frac{N}{10}$ sulphuric acid until the contents of the flask are nearly dry or until the distillate fails to give an alkaline reaction to litmus, showing the absence of ammonia. The time devoted to this process is ordinarily about an hour. Boil the distillate

FIG. 120.



DOREMUS-HINDS UREOMETER.

¹ If low results are obtained the heating should be continued one and one-half hours in subsequent determinations.

a few moments to free it from CO_2 , then cool and titrate the mixture with $\frac{N}{10}$ sodium hydroxide, using "alizarin red" as indicator.

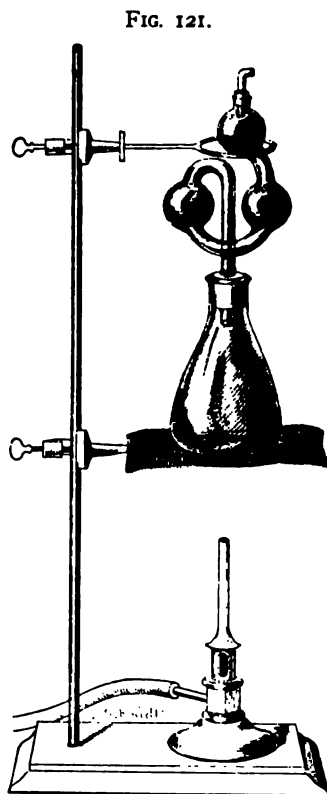
A "check" experiment should always be made to determine the original ammonia content of the urine and of the magne-

sium chloride, if it is not absolutely pure, which of course should be subtracted from the total amount of ammonia as determined by the above process.

The Folin method is extremely accurate under all conditions *except when the urine contains sugar*. When this is the case the carbohydrate and the urea unite, upon being heated, and form a very stable combination. For this reason the Folin method is not suitable for use in the examination of such urines. The best method for use under such conditions is the combination Mörner-Sjöqvist-Folin method which is given below.

4. **Mörner - Sjöqvist - Folin Method.**—As has already been stated in the last experiment this method excels the Folin method in accuracy *only* in the determination of urea in the

presence of carbohydrate bodies. Briefly the procedure is as follows:¹ Bring the major portion of 1.5 grams of powdered barium hydroxide into solution in 5 c.c. of urine in a small



FOLIN'S UREA APPARATUS.

¹The original description of the method may be found in an article by Mörner: *Skandinavisches Archiv für Physiologie*, 1903, xiv, p. 297.

flask, and treat the mixture with 100 c.c. of an alcohol-ether solution, consisting of two volumes of 97 per cent alcohol and one volume of ether. Stopper the flask and allow it to stand 12–24 hours. Filter off the precipitate, wash it with the alcohol-ether mixture and remove the alcohol and ether from the filtrate by distillation, being careful to keep the temperature of the mixture below 50°C .¹ Treat the remaining fluid (about 25 c.c.) with 2 c.c. of hydrochloric acid (sp. gr. 1.124), transfer it carefully to a 200 c.c. flask and evaporate the mixture to dryness on a water-bath. Now add 20 grams of crystallized magnesium chloride and 2 c.c. of concentrated hydrochloric acid to the residue and after fitting the flask with a return cooler boil the mixture on a wire gauze over a small flame for two hours. Cool the solution, dilute to 750 c.c. or to 1000 c.c. with water, render the mixture alkaline with potassium hydroxide or sodium hydroxide, distil off the ammonia and collect it in an acid solution of known strength. Boil the distillate to remove carbon dioxide, cool and titrate with an alkali of known strength. In this method, as well as in Folin's method (see p. 355), correction must be made for the ammonia originally present in the urine and in the magnesium chloride.

V. Ammonia.

1. **Folin's Method.**—Place 25 c.c. of urine in an ærometer cylinder, 30–45 cm. in height (Fig 122, p. 358), add about one gram of dry sodium carbonate and introduce some crude petroleum to prevent foaming. Insert into the neck of the cylinder a rubber stopper provided with two perforations into each of which passes a glass tube one of which reaches below the surface of the liquid. The shorter tube (10 cm. in length) is connected with a calcium chloride tube filled with cotton and this tube is in turn joined to a glass tube extending to the bottom of a 500 c.c. wide mouthed flask which is intended to absorb the ammonia and for this purpose should contain 20 c.c. of 16 sulphuric acid, 200 c.c. of distilled

¹ There is some decomposition of urea at 60°C .

water and a few drops of an indicator ("alizarin red"). To insure the complete absorption of the ammonia the absorption

FIG. 122.



FOLIN'S AMMONIA APPARATUS.

flask is provided with a Folin absorption tube (Fig. 123, p. 359) which is very effective in causing the air passing from the cylinder to come into intimate contact with the acid in the absorption flask. In order to exclude any error due to the presence of ammonia in the air a similar absorption apparatus to the one just described is attached to the other side of the aerometer cylinder, thus insuring the passage of *ammonia-free* air into the cylinder. With an ordinary filter pump and good water pressure the last trace of ammonia should be removed from the cylinder in about one and one-half hours.¹ The number of cubic centimeters of the $\frac{N}{10}$ sulphuric acid neutralized by the ammonia of the urine may be determined by direct titration with $\frac{N}{10}$ sodium hydroxide.

This is one of the most satisfactory methods yet devised for the determination of ammonia.

¹ With any given filter pump a "check" test should be made with urine or better with a solution of an ammonium salt of known strength to determine how long the air current must be maintained to remove all the ammonia from 25 c.c. of the solution.

Calculation.—Subtract the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide used in the titration from the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid taken. The remainder is the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid *neutralized by the NH_3 of the urine*. 1 c.c. of $\frac{N}{10}$ sulphuric acid is equivalent to 0.0017 gram of NH_3 . Therefore if y represents the volume of urine used in the determination and y' the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid *neutralized by the NH_3 of the urine*, we have the following proportion:

$$y:100::y' \times 0.0017:x \text{ (percentage of } NH_3 \text{ in the urine examined).}$$

Calculate the quantity of NH_3 in the twenty-four hour urine specimen.

VI. Nitrogen.

Kjeldahl Method.¹—The principle of this method is the conversion of the various nitrogenous bodies of the urine into ammonium sulphate by boiling with concentrated sulphuric acid, the subsequent decomposition of the ammonium sulphate by means of a fixed alkali (NaOH) and the collection of the liberated ammonia in an acid of known strength. Finally, this partly neutralized acid solution is titrated with an alkali of known strength and the nitrogen content of the urine under examination computed.

The procedure is as follows: Place 5 c.c. of urine in a 200–300 c.c. long-necked, Jena glass Kjeldahl digestion flask, add 20 c.c. of concentrated sulphuric acid and about 0.2 gram of cupric sulphate and boil the mixture for some time after

¹There are numerous modifications of the original Kjeldahl method; the one described here, however, has given excellent satisfaction and is recommended for the determination of the nitrogen content of urine.

FIG. 123.



FOLIN ABSORPTION TUBE.

it is colorless (about one hour). Allow the flask to cool and transfer¹ the contents, by means of about 200 c.c. of water, to a 750 c.c. Jena glass distillation flask. Add a little more of a concentrated solution of NaOH than is necessary to neutralize the sulphuric acid² and introduce into the flask a little coarse pumice stone or a few pieces of granulated zinc, to prevent bumping, and a small piece of paraffin to lessen the tendency to froth. By means of a safety-tube connect the flask with a condenser so arranged that the delivery-tube passes into a vessel containing a known volume (the volume used depending upon the nitrogen content of the urine) of $\frac{N}{10}$ sulphuric acid, using care that the end of the delivery-tube reaches beneath the surface of the fluid.³ Mix the contents of the distillation flask very thoroughly by shaking and distil the mixture until its volume has diminished about one-half. Titrate the partly neutralized $\frac{N}{10}$ sulphuric acid solution by means of $\frac{N}{10}$ sodium hydroxide, using congo red as indicator, and calculate the content of nitrogen of the urine examined.

Calculation.—Subtract the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide used in the titration from the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid taken. The remainder is equivalent to the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid, *neutralized by the ammonia of the urine*. One c.c. of $\frac{N}{10}$ sulphuric acid is equivalent to 0.0014 gram of nitrogen. Therefore, if y represents the volume of urine used in the determination, and y' the number of cubic centimeters of $\frac{N}{10}$ sulphuric acid *neutralized by the ammonia of the urine*, we have the following proportion:

¹A very satisfactory modification of this procedure includes the use of a 750 c.c. flask for both the digestion and the distillation, thus making unnecessary any transfer of contents.

²This concentrated sodium hydroxide solution should be prepared in quantity and "check" tests made to determine the volume of the solution necessary to neutralize the volume (20 c.c.) of concentrated sulphuric acid used.

³This delivery-tube should be of large caliber in order to avoid the "sucking back" of the fluid.

$y:100::y' \times 0.0014:x$ (percentage of nitrogen in the urine examined).

Calculate the quantity of nitrogen in the twenty-four hour urine specimen.

VII. Sulphur.

1. **Total Sulphates.**—*Folin's Method.*—Place 25 c.c. of urine in a 200–250 c.c. Erlenmeyer flask, add 20 c.c. of dilute hydrochloric acid¹ (1 volume of concentrated HCl to 4 volumes of water) and gently boil for 20–30 minutes. To minimize the loss of water by evaporation the mouth of the flask should be covered with a small watch glass during the boiling process. Cool the flask for 2–3 minutes in running water, and dilute the contents to about 150 c.c. by means of *cold* water. Add 10 c.c. of a 5 per cent solution of barium chloride slowly, drop by drop, to the cold solution.² The contents of the flask *should not be stirred or shaken* during the addition of the barium chloride. Allow the mixture to stand at least one hour, then shake up the solution and filter it through a weighed Gooch crucible.³

Wash the precipitate of BaSO_4 with about 250 c.c. of cold water, dry it in an air-bath or over a very low flame, then ignite,⁴ cool and weigh.

¹ If it is desired, 50 c.c. of urine and 4 c.c. of concentrated acid may be used instead.

² A dropper or capillary funnel made from an ordinary calcium chloride tube and so constructed as to deliver 10 c.c. in 2–3 minutes is recommended for use in adding the barium chloride.

³ If a Gooch crucible is not available the precipitate of BaSO_4 may be filtered off upon a washed filter paper (Schleicher & Schüll's, No. 589, blue ribbon) and after washing the precipitate with about 250 c.c. of *cold* water the paper and precipitate may be dried in an air-bath, or over a low flame. The ignition may then be carried out in the usual way in the ordinary platinum or porcelain crucible. In this case correction must be made for the weight of the ash of the filter paper used.

⁴ Care must be taken in the ignition of precipitates in Gooch crucibles. The flame should never be applied directly to the *perforated* bottom or to the sides of the crucible, since such manipulation is invariably attended by mechanical losses. The crucibles should always be provided with *lids* and *light bottoms* during the ignition. In case porcelain Gooch crucibles,

Calculation.—Subtract the weight of the Gooch crucible from the weight of the crucible and the BaSO_4 precipitate to obtain the weight of the precipitate. The weight of SO_3 in the volume of urine taken may be determined by means of the following proportion:

$$\begin{array}{ccccc} \text{Mol. wt.} & \text{Wt. of} & \text{Mol. Wt.} & & \\ \text{BaSO}_4 & : \text{BaSO}_4 & :: \text{SO}_3 & : x \text{ (wt. of SO}_3 \text{ in grams).} \\ & & & & \text{ppt.} \end{array}$$

Representing the weight of the BaSO_4 precipitate by y and substituting the proper molecular weights, we have the following proportion:

$$231.7 : y :: 79.5 : x \text{ (wt. of SO}_3 \text{ in grams in the quantity of urine used).}$$

Calculate the quantity of SO_3 in the twenty-four hour specimen of urine.

To express the result in *percentage* of SO_3 simply divide the value of x , as just determined, by the quantity of urine used.

2. Inorganic Sulphates.—*Folin's Method.*—Place 25 c.c. of urine and 100 c.c. of water in a 200–250 c.c. Erlenmeyer flask and acidify the diluted urine with 10 c.c. of dilute hydrochloric acid (1 volume of concentrated HCl to 4 volumes water). In case the urine is dilute 50 c.c. may be used instead of 25 c.c. and the volume of water reduced proportionately. From this point proceed as indicated in the method for the determination of Total Sulphates, page 361.

Calculate the quantity of inorganic sulphates, expressed as SO_3 , in the twenty-four hour urine specimen.

whose bottoms are not provided with a non-perforated cap, are used, the crucible may be placed upon the lid of an ordinary platinum crucible during ignition. The lid should be supported on a triangle, the crucible placed upon the lid and the flame applied to the improvised bottom. Ignition should be complete in 10 minutes if no organic matter is present.

¹ It is considered preferable by many investigators to express all sulphur values in terms of S rather than SO_3 .

Calculation.—Calculate according to the directions given under Total Sulphates, page 361.

3. **Ethereal Sulphates.**—*Folin's Method.*—Place 125 c.c. of urine in an Erlenmeyer flask of suitable size, dilute it with 75 c.c. of water and acidify the mixture with 30 c.c. of dilute hydrochloric acid (1 volume of concentrated HCl to 4 volumes of water). To the *cold* solution add 20 c.c. of a 5 per cent solution of barium chloride, drop by drop.¹ Allow the mixture to stand about one hour then filter it through a dry filter paper.² Collect 125 c.c. of the filtrate and boil it gently for at least one-half hour. Cool the solution, filter off the precipitate of BaSO₄, wash and ignite it according to the directions given on page 361.

Calculation.—The weight of the BaSO₄ precipitate should be multiplied by 2 since only one-half (125 c.c.) of the total volume (250 c.c.) of fluid was precipitated by the barium chloride. The remaining calculation should be made according to directions given under Total Sulphates, page 361.

Calculate the quantity of ethereal sulphates, expressed as SO₃, in the twenty-four hour urine specimen.

4. **Total Sulphur.**—*Osborne-Folin Method.*—Place 25 c.c. of urine³ in a 200-250 c.c. *nickel* crucible and add about 3 grams of sodium peroxide. Evaporate the mixture to a syrup and heat it carefully until it solidifies (15 minutes). Now remove the crucible from the flame and allow it to cool. Moisten the residue with 1-2 c.c. of water,⁴ sprinkle about 7-8 grams of sodium peroxide over the contents of the crucible and fuse the mass for about 10 minutes. Allow the

¹ See note (2) at the bottom of page 361.

² This precipitate consists of the inorganic sulphates. If it is desired, this BaSO₄ precipitate may be collected in a Gooch crucible or on an ordinary quantitative filter paper and a determination of inorganic sulphates made, using the same technique as that suggested on p. 361. In this way we are enabled to determine the inorganic and ethereal sulphates in the same sample of urine.

³ If the urine is very dilute 50 c.c. may be used.

⁴ This moistening of the residue with a small amount of water is very essential and should not be neglected.

crucible to cool for a few minutes, add about 100 c.c. of water to the contents and heat at least one-half hour, to dissolve the alkali and decompose the sodium peroxide. Next rinse the mixture into a 400-450 c.c. Erlenmeyer flask, by means of hot water, and dilute it to about 250 c.c. Heat the solution nearly to the boiling-point and add concentrated hydrochloric acid slowly until the nickelic oxide, derived from the crucible, is just brought into solution.¹ A few minutes boiling should now yield a clear solution. In case too little peroxide or too much water was added for the final fusion a clear solution will not be obtained. In this event cool the solution and remove the insoluble matter by filtration.

To the clear solution add 5 c.c. of very dilute alcohol (about 18-20 per cent) and continue the boiling for a few minutes. The alcohol is added to remove the chlorine which was formed when the solution was acidified. Add 10 c.c. of a 10 per cent solution of barium chloride, slowly, drop by drop,² to the liquid. Allow the precipitated solution to stand in the crucible two days and then filter and continue the manipulation according to the directions given under Total Sulphates, page 361.

Calculation.—Make the calculation according to the directions given under Total Sulphates, p. 361. Calculate the quantity of sulphur, expressed as SO_3 or S, present in the twenty-four hour urine specimen.

5. Total Sulphur.—*Sodium Hydroxide and Potassium Nitrate Fusion Method.*—Place 25 c.c. of urine in a silica crucible and evaporate to a thick syrup on a water-bath. Add 10 grams of sodium hydroxide and 2 grams of potassium nitrate to the residue and fuse the mass, over an alcohol flame until all organic matter has disappeared and the fused mixture is clear. Cool the mixture, transfer it to a casserole, by means of hot water, acidify slightly with hydrochloric acid and evaporate it to dryness on a water-bath. Moisten the residue with a few drops of dilute hydrochloric acid and bring it in

¹ About 18 c.c. of acid is required for 8 grams of sodium peroxide.

² See note (2) at the bottom of page 361.

solution with hot water. Filter, heat the filtrate to boiling and immediately precipitate it by the addition of 10 c.c. of a 10 per cent solution of barium chloride, adding the solution slowly, drop by drop. Allow the precipitated solution to stand 2 hours and filter while *cold*. Ignite, weigh and calculate according to directions given under Total Sulphates, p. 361.

Compute the quantity of sulphur, expressed as SO_3 or S, present in the twenty-four hour urine specimen.

6. Total Sulphur.—*Sherman's Compressed Oxygen Method.*¹ Evaporate as much urine on an absorbent filter block² at 60°C . as the block will conveniently absorb and burn the block so prepared in a bomb-calorimeter³ using 25–30 atmospheres of oxygen. Connect the bomb with a wash-bottle containing water, and allow the gas to bubble through the liquid until the high pressure within the apparatus has been reduced to atmospheric pressure. Now open the bomb and thoroughly rinse the interior, using water from the wash-bottle for the first rinsing. Dissolve any ash found in the combustion capsule in hydrochloric acid and add this solution to the main solution. Evaporate to 150 c.c., filter and cool the filtrate. Add 10 c.c. of a 5 per cent solution of barium chloride to the *cold* filtrate, slowly, drop by drop.⁴ The contents of the flask should not be stirred or shaken during the addition of the barium chloride. Allow the mixture to stand at least one hour, then shake up the solution and filter it through a weighed Gooch crucible. Manipulate the precipitate of BaSO_4 according to directions given under Total Sulphates, page 361.

Calculate the quantity of sulphur, expressed as SO_3 or S, present in the twenty-four hour urine specimen.

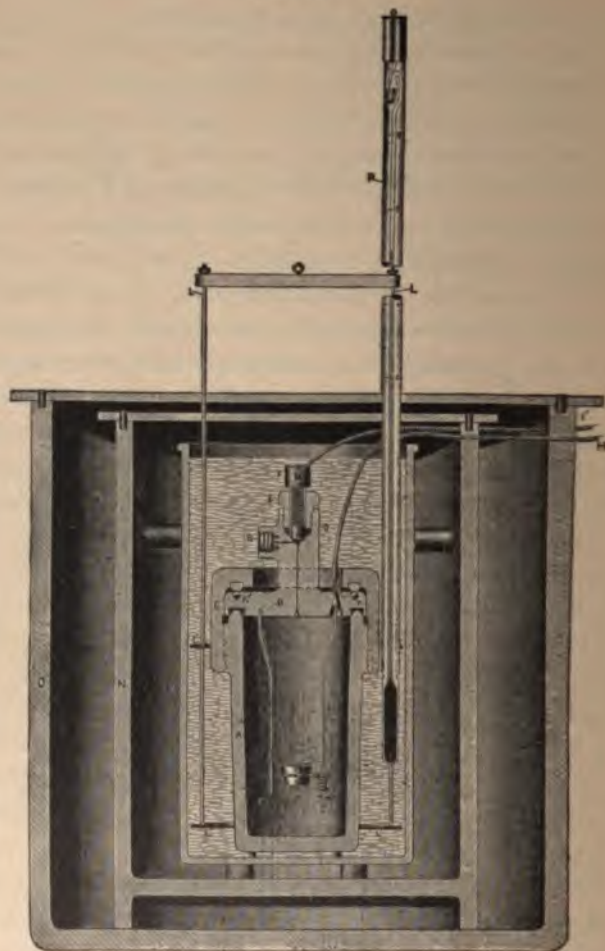
¹ See Sherman's Organic Analysis, p. 19.

² Only a small amount of urine should be added at one time, it being necessary to make several evaporations before the block contains sufficient urinary residue to proceed with the combustion.

³ The Berthelot-Atwater apparatus (Fig. 124, page 366) is well adapted to this purpose.

⁴ See note (2) at the bottom of page 361.

FIG. 124.



BERTHELOT-ATWATER BOMB CALORIMETER. (CROSS-SECTION OF APPARATUS AS READY FOR USE.)

A, Steel cup or bomb proper; C, collar of steel; G, opening through which oxygen is forced into the bomb; H and I', insulated wires which serve to conduct an electric current for igniting the substance which is held in the small capsule B; L, a stirrer which serves to keep the water surrounding the bomb in motion and insures the equalization of temperature; P, a delicate thermometer which serves to measure the rise in temperature of the water surrounding the bomb.

VIII. Phosphorus.

1. **Total Phosphates.**—*Uranium Acetate Method.*—To 50 c.c. of urine in a small beaker or Erlenmeyer flask add 5 c.c. of a special sodium acetate solution¹ and heat the mixture to the boiling-point. From a burette, run into the hot mixture, drop by drop, a standard solution of uranium acetate² until a precipitate ceases to form and a drop of the mixture when removed by means of a glass rod and brought in contact with a drop of a solution of potassium ferrocyanide on a porcelain test-tablet produces instantaneously a brownish-red coloration.³ Take the burette reading and calculate the P_2O_5 content of the urine under examination.

Calculation.—Multiply the number of cubic centimeters of uranium acetate solution used by 0.005 to determine the number of grams of P_2O_5 in the 50 c.c. of urine used. To express the result in *percentage* of P_2O_5 multiply the value just obtained by 2, *e. g.*, if 50 c.c. of urine contained 0.074 gram of P_2O_5 it would be equivalent to 0.148 per cent.

Calculate, in terms of P_2O_5 , the total phosphate content of the twenty-four hour urine specimen.

2. **Earthy Phosphates.**—To 100 c.c. of urine in a beaker

¹ The sodium acetate solution is prepared by dissolving 100 grams of sodium acetate in 800 c.c. of distilled water, adding 100 c.c. of 30 per cent acetic acid to the solution and making the volume of the mixture up to 1 liter with water.

² This uranium acetate solution may be prepared by dissolving 35.461 grams of uranium acetate in one liter of water. One c.c. of such a solution should be equivalent to 0.005 gram of P_2O_5 , phosphoric anhydride. This solution may be standardized as follows: To 50 c.c. of a standard solution of disodium hydrogen phosphate, of such a strength that the 50 c.c. contains 0.1 gram of P_2O_5 , add 5 c.c. of the sodium acetate solution, mentioned above, and titrate with the uranium solution to the correct end-reaction as indicated in the method proper. Inasmuch as 1 c.c. of the uranium solution should precipitate 0.005 gram of P_2O_5 , exactly 20 c.c. of the uranium solution should be required to precipitate 50 c.c. of the standard phosphate solution. If the two solutions do not bear this relation to each other they may be brought into proper relation by diluting the uranium solution with distilled water or by increasing its strength.

³ A 10 per cent solution of potassium ferrocyanide is satisfactory.

add an excess of ammonium hydroxide and allow the mixture to stand 12–24 hours. Under these conditions the phosphoric acid in combination with the alkaline earths, calcium and magnesium, is precipitated as phosphates of these metals. Collect the precipitate on a filter paper and wash it with very dilute ammonium hydroxide. Pierce the paper, and remove the precipitate by means of hot water. Bring the phosphate into solution by adding a small amount of dilute acetic acid to the warm solution. Make the volume up to 50 c.c. with water, add 5 c.c. of sodium acetate solution and determine the P_2O_5 content of the mixture according to the direction given under the previous method.

Calculation.—Multiply the number of cubic centimeters of uranium acetate solution used by 0.005 to determine the number of *grams* of P_2O_5 in the 100 c.c. of urine used. Since 100 c.c. of urine was taken this value also expresses the *percentage* of P_2O_5 present.

Calculate the quantity of earthy phosphates, in terms of P_2O_5 , present in the twenty-four hour urine specimen.

The quantity of phosphoric acid present in combination with the *alkali* metals may be determined by subtracting the content of earthy phosphates from the total phosphates.

3. **Total Phosphorus.**—*Sodium Hydroxide and Potassium Nitrate Fusion Method.*—Place 25 c.c. of urine in a large silver crucible and evaporate to a syrup on a water-bath. Add 10 grams of NaOH and 2 grams of KNO_3 to the residue and fuse the mass until all organic matter has disappeared and the fused mixture is clear. Cool the mixture, transfer it to a casserole by means of hot water, acidify the solution slightly with pure nitric acid and evaporate to dryness on a water-bath. Moisten the residue with a few drops of dilute nitric acid, dissolve it in hot water and transfer to a beaker. Now add an equal volume of molybdic solution¹ and keep the mixture at 40° C. for twenty-four hours. Filter off the precipitate, wash it with dilute molybdic solution and dissolve it in dilute am-

¹ Directions for the preparation of the solution are given on page 37.

monia. Add dilute hydrochloric acid to the solution, being careful to leave the solution distinctly ammoniacal. Magnesia mixture² (10–15 c.c.) should now be added and after stirring thoroughly and making strongly ammoniacal with concentrated ammonia the solution should be allowed to stand in a cool place for twenty-four hours. Filter off the precipitate, wash it free from chlorine by means of dilute ammonia (1:5), dry, incinerate and weigh, as magnesium pyrophosphate, $\text{Mg}_2\text{P}_2\text{O}_7$, in the usual manner.

In this method the phosphoric acid of the urine is precipitated as *ammonium magnesium phosphate* and in the process of incineration this body is transformed into *magnesium pyrophosphate*.

Calculation.—The quantity of phosphorus, expressed in terms of P_2O_5 , in the volume of urine taken may be determined by means of the following proportion:

$$\begin{array}{ccccc} \text{Mol. wt.} & & \text{Wt. of} & & \text{Mol. wt.} \\ \text{Mg}_2\text{P}_2\text{O}_7 & : & \text{Mg}_2\text{P}_2\text{O}_7 & : : & \text{P}_2\text{O}_5 : x \text{ (wt. of } \text{P}_2\text{O}_5 \text{ in grams).} \\ & & \text{ppt.} & & \end{array}$$

If y represents the weight of the $\text{Mg}_2\text{P}_2\text{O}_7$ precipitate and we make the proper substitutions we have the following proportion:

$$221.1 : y :: 140.9 : x \text{ (wt. of } \text{P}_2\text{O}_5 \text{, in grams, in the quantity of urine used).}$$

To express the result in *percentage* of P_2O_5 simply divide the value of x , as just determined, by the quantity of urine used.

IX. Creatinin.

Folin's Colorimetric Method.—This method is based upon the characteristic property possessed alone by creatinin, of yielding a certain definite color-reaction in the presence of picric acid in alkaline solution. The procedure is as follows:

²Directions for the preparation of magnesia mixture may be found on page 270.

Place 10 c.c. of urine in a 500 c.c. volumetric flask, add 15 c.c. of a *saturated* solution of picric acid and 5 c.c. of a 10 per cent solution of sodium hydroxide and allow the mixture to stand for 5-6 minutes. During this interval pour a little $\frac{N}{2}$ potassium bichromate solution¹ into each of the two cylinders of the colorimeter (Duboscq's) and carefully adjust the depth of the solution in one of the cylinders to the 8 mm. mark. A few preliminary colorimetric readings may now be made with the solution in the other cylinder, in order to insure greater accuracy in the subsequent examination of the solution of unknown strength. Obviously the two solutions of potassium bichromate are identical in color and in their examination no two readings should differ more than 0.1-0.2 mm. from the true value (8 mm.). Four or more readings should be made in each case and an average taken of all of them *exclusive* of the first reading, which is apt to be less accurate than the succeeding readings. In time as one becomes proficient in the technique it is perfectly safe to take the average of the *first two readings*.

At the end of the 5-6 minute interval already mentioned, the contents of the 500 c.c. flask are diluted to the 500 c.c. mark, the bichromate solution is thoroughly rinsed out of one of the cylinders with the solution thus prepared and a number of colorimetric readings are *immediately* made.

Ordinarily 10 c.c. of urine is used in the determination by this method but if the content of creatinin is above 15 mg. or below 5 mg. the determination should be repeated with a volume of urine selected according to the content of creatinin. This variation in the volume of urine according to the content of creatinin is quite essential since the method loses in accuracy when more than 15 mg. or less than 5 mg. of creatinin is present in the solution of unknown strength.

Calculation.—By experiment it has been determined that 10 mg. of pure creatinin, when brought into solution and diluted to 500 c.c. as explained in the above method, yields a mixture 8.1 mm. of which possesses the same colorimetric

¹This solution contains 24.55 gr. potassium bichromate to the liter.

value as 8 mm. of a $\frac{N}{2}$ solution of potassium bichromate. Bearing this in mind the computation is readily made by means of the following proportion in which y represents the number of mm. of the solution of unknown strength equivalent to the 8 mm. of the potassium bichromate solution:

$y:8.1::10:x$ (mgs. of creatinin in the quantity of urine used).

This proportion may be used for the calculation no matter what volume of urine (5, 10 or 15 c.c.) is used in the determination. The 10 represents 10 mg. of creatinin, which gives a color equal to 8.1 mm., whether dissolved in 5, 10 or 15 c.c. of fluid.

Calculate the quantity of creatinin in the twenty-four hour urine specimen.

X. Chlorides.

1. **Mohr's Method.**—To 10 c.c. of urine in a small platinum or porcelain crucible or dish add about 2 grams of chlorine-free potassium nitrate and evaporate to dryness at 100° C. (The evaporation may be conducted over a low flame provided care is taken to prevent loss by spurting.) By means of crucible tongs hold the crucible or dish over a free flame until all carbonaceous matter has disappeared and the fused mass is slightly yellow in color. Cool the residue somewhat and bring it into solution in a small amount (15–25 c.c.) of distilled water acidified with about 10 drops of nitric acid. Transfer the solution to a small beaker, being sure to rinse out the crucible or dish very carefully. Test the reaction of the fluid, and if not already acid in reaction, to litmus, render it slightly acid with nitric acid. Now neutralize the solution by the addition of calcium carbonate in substance,¹ add 2–5 drops of neutral potassium chromate solution to the mixture and titrate with a standard argentic nitrate solution.²

¹ The cessation of effervescence and the presence of some undecomposed calcium carbonate at the bottom of the vessel are the indications of neutralization.

² Standard argentic nitrate solution may be prepared by dissolving 29.060 grams of argentic nitrate in 1 liter of distilled water. Each cubic

This standard solution should be run in from a burette stirring the liquid in the beaker after each addition. The end reaction is reached when the yellow color of the solution changes to a slight *orange-red*. At this point take the buret reading and compute the percentage of chlorine and sodium chloride in the urine examined.

Calculation.—Since 1 c.c. of the standard argentic nitrate solution is equivalent to 0.010 gram of sodium chloride, to obtain the *weight*, in grams, of the *sodium chloride* in the 1 c.c. of urine used multiply the number of cubic centimeters of standard solution used by 0.010. If it is desired to express the result in *percentage* of sodium chloride move the decimal point *one* place to the *right*.

To obtain the *weight*, in grams, of the *chlorine* in the 10 c.c. of urine used multiply the number of cubic centimeters of standard solution used by 0.006, and if it is desired to express the result in *percentage* of chlorine move the decimal point *one* place to the *right*.

Calculate the quantity of sodium chloride and chlorine in the twenty-four hour urine specimen.

2. **Volhard-Arnold Method.**—Place 10 c.c. of urine in 100 c.c. volumetric flask, add 20-30 drops of nitric acid (sp. g. 1.2) and 2 c.c. of a cold saturated solution of ferric alum. If necessary, at this point a few drops of an 8 per cent solution of potassium permanganate may be added to dissipate the red color. Now slowly run in the standard argentic nitrate¹ solution (20 c.c. is ordinarily used) until all the chlorine has been precipitated and an excess of the argentic nitrate solution is present, continually shaking the mixture during the addition of the standard solution. Allow the flask to stand 10 minutes then fill it to the 100 c.c. graduation with distilled water and *thoroughly mix* the contents. Now filter the mixture through a dry filter paper, collect 50 c.c. of the filtrate and titrate it with

centimeter of this solution is equivalent to 0.010 gram of sodium chloride or to 0.006 gram of chlorine.

¹ See note (2) at the bottom of page 371.

standardized ammonium sulphocyanide solution.¹ The first permanent tinge of brown indicates the end-point. Take the burette reading and compute the weight of sodium chloride in the 10 c.c. of urine used.

Calculation.—The number of cubic centimeters of ammonium sulphocyanide solution used indicates the excess of standard argentic nitrate solution in the 50 c.c. of filtrate titrated. Multiply this reading by 2, inasmuch as only one-half of the filtrate was employed, and subtract this product from the number of cubic centimeters of argentic nitrate (20 c.c.) originally used, in order to obtain the actual number of cubic centimeters of argentic nitrate utilized in the precipitation of the chlorides in the 10 c.c. of urine employed.

To obtain the weight, in grams, of the sodium chloride in the 10 c.c. of urine used multiply the number of cubic centimeters of the standard argentic nitrate solution, actually utilized in the precipitation, by 0.010. If it is desired to express the result in *percentage* of sodium chloride move the decimal point *one* place to the *right*.

In a similar manner the weight, or percentage of *chlorine* may be computed using the factor 0.006 as explained in Mohr's method, page 371.

Calculate the quantity of sodium chloride and chlorine in the twenty-four hour urine specimen.

¹This solution is made of such a strength that 1 c.c. of it is equal to 1 c.c. of the standard argentic nitrate solution used. To prepare the solution dissolve 12.9 grams of ammonium sulphocyanide, NH_4SCN , in a little less than a liter of water. In a small flask place 20 c.c. of the standard argentic nitrate solution, 5 c.c. of the ferric alum solution and 4 c.c. of nitric acid (sp. gr. 1.2), add water to make the total volume 100 c.c. and thoroughly mix the contents of the flask. Now run in the ammonium sulphocyanide solution from a burette until a permanent brown tinge is produced. This is the end-reaction and indicates that the last trace of argentic nitrate has been precipitated. Take the burette reading and calculate the amount of water necessary to use in diluting the ammonium sulphocyanide in order that 10 c.c. of this solution may be exactly equal to 10 c.c. of the argentic nitrate solution. Make this dilution and titrate again to be certain that the solution is of the proper strength.

XI. Acetone.

Messinger-Huppert Method.—Place 100 c.c. of urine in a distillation flask and add 2 c.c. of 50 per cent acetic acid. Connect the flask with a condenser, properly arrange a receiver, attach a terminal series of bulbs containing water and distil over about nine-tenths of the urine mixture. Remove the receiver, attach another and subject the residual portion of the mixture to a second distillation. Test this fluid for acetone and if the presence of acetone is indicated add about 100 c.c. of water to the residue and again distil. Treat the united acetone distillates with 1 c.c. of dilute (12 per cent) sulphuric acid and redistil, collecting this second distillate in a glass-stoppered flask. During distillation, however, the glass stopper is replaced by a cork with a double perforation, the glass tube from one perforation passing to the condenser while the bulbs containing water, before-mentioned, are attached by means of the tube in the other perforation. Allow the distillation process to proceed until practically all of the fluid has passed over, then remove the receiving flask and insert the glass stopper. Now treat the distillate carefully with 10 c.c. of a $\frac{N}{10}$ solution of iodine and add sodium hydroxide solution, drop by drop, until the blue color is dissipated and the iodoform precipitates. Stopper the flask and shake it for about one minute, acidify the solution with concentrated hydrochloric acid, and note the production of a brown color if an excess of iodine is present. In case there is no such excess, the solution should be treated with $\frac{N}{10}$ iodine solution until an excess is obtained. Retitrate this excess of iodine with $\frac{N}{10}$ sodium thiosulphate solution until a light yellow color is observed. At this point a few cubic centimeters of starch paste should be added and the mixture again titrated until no blue color is visible. This is the end-reaction.

Calculation.—Subtract the number of cubic centimeters of $\frac{N}{10}$ thiosulphate solution used from the volume of $\frac{N}{10}$ iodine solution employed. Since 1 c.c. of the iodine solution is equivalent to 0.967 milligrams of acetone, and since 1 c.c. of

the thiosulphate solution is equivalent to 1 c.c. of the iodine solution, if we multiply the remainder from the above subtraction by 0.967 we will obtain the number of milligrams of acetone in the 100 c.c. of urine examined.

Calculate the quantity of acetone in the twenty-four hour urine specimen.

XII. β -Oxybutyric Acid.

1. **Darmstädter's Method.**—This method is based on the fact that crotonic acid is formed from β -oxybutyric acid under the influence of concentrated mineral acids. The method is as follows: Render 100 c.c. of urine slightly alkaline with sodium carbonate and evaporate nearly to dryness on a water-bath. Dissolve the residue in 150–200 c.c. of 50–55 per cent sulphuric acid, transfer the acid solution to a one liter distillation flask and connect it with a condenser. Through the cork of the flask introduce the stem of a dropping funnel containing water. Heat the flask gently until foaming ceases, then use a full flame and distil over about 300–350 c.c. of fluid, keeping the volume of liquid in the distillation flask constant by the addition of water from the dropping funnel as the distillate collects. Ordinarily it will take about 2–2½ hours to collect this amount of distillate. Extract the distillate two or three times with ether in a separatory funnel, evaporate the ether and heat the residue at 160° C. for a few minutes to remove volatile fatty acids. Dissolve the residue in 50 c.c. of water, filter and titrate this aqueous solution of crotonic acid with $\frac{N}{10}$ sodium hydroxide solution, using phenolphthalein as indicator.

Calculation.—One c.c. of $\frac{N}{10}$ sodium hydroxide solution equals 0.0086 gram of crotonic acid, 1 part of crotonic acid equals 1.21 part of β -oxybutyric acid, and 1 c.c. of $\frac{N}{10}$ sodium hydroxide solution equals 0.01041 gram of β -oxybutyric acid. To compute the quantity of β -oxybutyric acid, in grams, multiply the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide solution used by 0.01041.

2. **Bergell's Method.**—Render 100–300 c.c. of sugar-free¹ urine slightly alkaline with sodium carbonate, evaporate the alkaline urine to a syrup on a water-bath, cool the syrup, rub it up with syrupy phosphoric acid (being careful to keep the mixture cool), 20–30 grams of finely pulverized, anhydrous cupric sulphate and 20–25 grams of fine sand. Mix the mass thoroughly, place it in a paper extraction thimble² and extract the dry mixture with ether in a Soxhlet apparatus (Fig. 12; page 380). Evaporate the ether, dissolve the residue in about 25 c.c. of water, decolorize the fluid with animal charcoal, if necessary, and determine the content of β -oxybutyric acid by a polarization test.

3. **Boekelman and Bouma's Method.**—Place 25 c.c. of urine in a flask, add 25 c.c. of 12 per cent sodium hydroxide and 25 c.c. of benzoyl chloride, stopper the flask and shake it vigorously for three minutes *under cold water*. Remove the clear fluid by means of a pipette, filter it and subject it to a polarization test. Through the action of the benzoyl chloride all the levorotatory substances except β -oxybutyric acid will have been removed and the levorotation now exhibited by the urine will be due entirely to that acid.

XIII. Acidity.

Folin's Method.—The *total acidity* of urine may be determined as follows: Place 25 c.c. of urine in a 200 c.c. Erlenmeyer flask and add 15–20 grams of finely pulverized potassium oxalate and 1–2 drops of a 1 per cent phenolphthalein solution to the fluid. Shake the mixture vigorously for 1–2 minutes and titrate it immediately with $\frac{N}{10}$ sodium hydroxide until a *permanent faint pink* coloration is produced. Take the burette reading and calculate the acidity of the urine under examination.

Calculation.—If y represents the number of cubic centi-

¹ If sugar is present it must be removed by fermentation.

² The Schleicher and Schüll fat-free extraction thimble is very satisfactory.

meters of $\frac{N}{10}$ sodium hydroxide used and y' represents the volume of urine excreted in twenty-four hours, the *total acidity* of the twenty-four hour urine specimen may be calculated by means of the following proportion:

$25 : y :: y' : x$ (acidity of 24-hour urine expressed in cubic centimeters of $\frac{N}{10}$ sodium hydroxide).

Each cubic centimeter of $\frac{N}{10}$ sodium hydroxide contains 0.004 gram of sodium hydroxide and this is equivalent to 0.0063 gram of oxalic acid. Therefore, in order to express the total acidity of the twenty-four hour urine specimen in equivalent grams of sodium hydroxide, multiply the value of x , as just determined, by 0.004, or multiply the value of x by 0.0063 if it is desired to express the total acidity in grams of oxalic acid.

XIV. Purin Bases.

Salkowski's Method.—Place 400–600 c.c. of proteid-free urine in a beaker. Introduce into another beaker 30–50 c.c. of an ammoniacal silver solution¹ with 30–50 c.c. of magnesia mixture,² add some ammonium hydroxide and if necessary some ammonium chloride to clear the solution. Now add this solution to the urine, stirring continually with a glass rod, and allow the mixture to stand for one-half hour. Collect the precipitate on a filter paper, wash it with dilute ammonium hydroxide and finally wash it back into the original beaker. Suspend the precipitate in 600–800 c.c. of water, add a few drops of hydrochloric acid and decompose it by means of hydrogen sulphide. Now heat the solution to boiling, filter while hot and evaporate the filtrate to dryness on a water-bath. Extract the residue with 20–30 c.c. of hot 3 per cent sulphuric acid and allow the extract to stand twenty-four hours. Filter off the uric acid, wash it, make the filtrate

¹ Prepared by dissolving 26 grams of silver nitrate in about 500 c.c. of water, adding enough ammonium hydroxide to redissolve the precipitate which forms upon the first addition of the ammonia and making the balance of the mixture up to 1 liter with water.

² Directions for preparation may be found on page 270.

ammoniacal and precipitate the purin bases again with silver nitrate. Collect this precipitate on a small-sized chlorine-free filter paper, wash, dry and incinerate it in the usual manner. Now dissolve the ash in nitric acid and titrate with ammonium sulphocyanide according to the Volhard-Arnold method (see 372). Calculate the content of purin bases in the urine examined, bearing in mind that in an equal mixture of the silver salts of the purin bases, such as we have here, one part of silver corresponds to 0.277 gram of nitrogen or to 0.7381 gram of the bases.

XV. Oxalic Acid.

Salkowski-Autenrieth and Barth Method.—Place the twenty-four hour urine specimen in a precipitating jar, add an excess of calcium chloride, render the urine strongly ammoniacal, stir it well and allow it to stand 18–20 hours. Filter off the precipitate, wash it with a small amount of water and dissolve it in about 30 c.c. of a hot 15 per cent solution of hydrochloric acid. By means of a separator funnel extract the solution with 150 c.c. of ether which contains 3 per cent of alcohol, repeating the extraction four or five times with fresh portions of ether. Unite the ethereal extracts, allow them to stand for an hour in a flask and then filter through a dry filter paper. Add 5 c.c. of water to the filtrate, to prevent the formation of diethyl oxalate when the solution is heated, and distil off the ether. If necessary decolorize the liquid with animal charcoal and filter. Concentrate the filtrate to 3–5 c.c., add a little calcium chloride solution, make it ammoniacal and after a few minutes render it slightly acid with acetic acid. Allow the acidified solution to stand several hours, collect the precipitate of calcium oxalate on a washed filter paper,¹ wash, incinerate strongly (to CaO) and weigh in the usual manner.

Calculation.—Since 56 parts of CaO are equivalent to 90 parts of oxalic acid, the quantity of oxalic acid in the volume

¹ Schleicher and Schüll, No. 589, is satisfactory.

of urine taken may be determined by multiplying the weight of CaO by the factor 1.6071.

XVI. Total Solids.

1. **Drying Method.**—Place 5 c.c. of urine in a weighed shallow dish, acidify *very slightly* with acetic acid (1–3 drops) and dry it *in vacuo* in the presence of sulphuric acid, to constant weight. Calculate the *percentage* of solids in the urine sample and the *total solids* for the twenty-four hour period.

Practically all the methods the technique of which includes evaporation at an increased temperature, either under atmospheric conditions or *in vacuo* are attended with error.

2. **Calculation by Long's Coefficient.**—The quantity of solid material contained in the urine excreted for any twenty-four hour period may be approximately computed by multiplying the second and third decimal figures of the specific gravity by 2.6. This gives us the *number of grams of solid matter in one liter of urine*. From this value the total solids for the twenty-four hour period may easily be determined.

Calculation.—If the volume of urine for the twenty-four hours was 1120 c.c. and the specific gravity 1.018, the calculation would be as follows:

(a) $18 \times 2.6 = 46.8$ grams of solid matter in 1 liter of urine.

(b) $\frac{46.8 \times 1120}{1000} = 52.4$ grams solid matter in 1120 c.c. of urine.

Long's coefficient was determined for urine whose specific gravity was taken at 25° C. and is probably more accurate, for conditions obtaining in America, than the older coefficient of Haeser, 2.33.

CHAPTER XXII.

QUANTITATIVE ANALYSIS OF MILK, GASTRIC JUICE AND BLOOD.

(a) Quantitative Analysis of Milk.

1. **Specific Gravity.**—This may be determined conveniently by means of a Soxhlet, Veith or Quevenne *lactometer*. A lactometer reading of 32° denotes a specific gravity of

FIG. 125.



SOXHLET APPARATUS.

1.032. The determination should be made at about 60° F. and the lactometer reading corrected by adding or subtracting 0.1 for every degree F. above or below that temperature.

2. **Fat.**—(a) *Adams' Paper Coil Method.*—Introduce about 5 c.c. of milk into a small beaker, quickly ascertain the weight to centigrams, stand a fat-free paper coil¹ in the beaker and incline the vessel and rotate the coil in order to hasten the absorption of the milk. Immediately upon the complete absorption of the milk remove the coil and again quickly ascertain the weight of the beaker. The difference in the weights of the beaker at the two weighings represents the quantity of milk absorbed by the coil. Dry the coil carefully at a temperature below 100° C. and extract with ether for 3–5 hours in

¹Very satisfactory coils are manufactured by Schleicher and Schüll.

Soxhlet apparatus (Fig. 125, p. 380), using a safety water-bath, Heat the flask containing the fat to constant weight at a temperature below 100° C.

Calculation.—Divide the weight of fat, in grams, by the weight of milk, in grams. The quotient is the *percentage of fat* contained in the milk examined.

(b) *Approximate Determination by Feser's Lactoscope.*—Milk is opaque mainly because of the suspended fat globules and therefore by means of the estimation of this opacity we may obtain data as to the *approximate* content of fat. Feser's lactoscope (Fig. 126, p. 381) may be used for this purpose. Proceed as follows: By means of the graduated pipette accompanying the instrument introduce 4 c.c. of milk into the lactoscope. Add water gradually, shaking after each addition, and note the point at which the black lines upon the inner white glass cylinder are *distinctly* visible. Observe the point on the graduated scale of the lactoscope which is level with the surface of the diluted milk. This reading represents the *percentage of fat present in the undiluted milk*. Pure milk should contain at least 3 per cent of fat.

FIG. 126.

FESER'S
LACTOSCOPE.

3. **Total Solids.**¹—Introduce 2–5 grams of milk into a *weighed* flat-bottomed platinum dish and quickly ascertain the weight to milligrams. Expel the major portion of the water by heating the *open* dish on a water-bath and continue the heating in an air-bath or water oven at 97°–100° C. until the weight is constant. (This residue may be used in the determination of *ash* according to the method described on p. 382.)

¹ The percentage of total solids may be calculated from the *specific gravity* and *percentage of fat* by means of the following formula which has been proposed by Richmond:

$$S = 0.25 L + 1.2 F + 0.14$$

S = total solids.

L = lactometer reading.

F = fat content.

Calculation.—Divide the weight of the residue, in grams, by the weight of milk used, in grams. The quotient is the *percentage of solids* contained in the milk examined.

4. **Ash.**—Heat the dry solids from 2–5 grams of milk obtained according to the method just given, over a very low flame¹ until a white or light gray ash is obtained. Cool the dish in a desiccator and weigh. (This ash may be used in testing for preservatives according to directions on page 195.)

5. **Proteids.**—Introduce a known weight of milk (5–10 grams) into a 200–300 c.c. Kjeldahl digestion flask and add 20 c.c. of concentrated sulphuric acid and about 0.2 gram of cupric sulphate. Expel the major portion of the water by heating over a low flame and finally use a full flame and allow the mixture to boil 1–2 hours. Complete the determination according to the directions given under Kjeldahl Method, page 359.

Calculation.—Multiply the *total nitrogen* content by the factor 6.37² to obtain the proteid content of the milk examined.

6. **Casein.**—Mix about 20 grams of milk with 40 c.c. of a saturated solution of magnesium sulphate and add the same substance until no more will dissolve. The precipitate consists of casein admixed with a little fat and lactoglobulin. Filter off the precipitate, wash it thoroughly with a saturated solution of magnesium sulphate,³ transfer the filter paper and precipitate to a Kjeldahl digestion flask and determine the nitrogen content according to the directions given in the previous experiment.

Calculation.—Multiply the *total nitrogen* by the factor 6.37 to obtain the casein content.

¹Great care should be used in this ignition, the dish at no time being heated above a faint redness, as chlorides may volatilize.

²The usual factor employed for the calculation of proteid from the nitrogen content is 6.25 and is based on the assumption that proteids contain on the average 16 per cent of nitrogen. This special factor of 6.37 is used here to calculate the proteid content from the total nitrogen since the principal proteid constituents of milk, *i. e.*, casein and lactalbumin contain 15.7 per cent of nitrogen.

³Preserve the filtrate and washings for the determination of lactalbumin

7. **Lactalbumin.**—To the filtrate and washings from the determination of casein, as just explained, add Almén's reagent¹ until no more precipitate forms. Filter off the precipitate and determine the nitrogen content according to the directions given under Proteids, page 382.

Calculation.—Multiply the *total nitrogen* by the factor 6.37 to obtain the lactalbumin content.

8. **Lactose.**—To about 350 c.c. of water in a beaker add 20 grams of milk, mix thoroughly, acidify the fluid with about 2 c.c. of 10 per cent acetic acid and stir the acidified mixture continuously until a flocculent precipitate forms. At this point the reaction should be distinctly acid to litmus. Heat the solution to boiling for one-half hour, filter, rinse the beaker thoroughly and wash the precipitated proteids and the adherent fat with *hot* water. Combine the filtrate and wash water and concentrate the mixture to about 150 c.c. Cool the solution and dilute it to 200 c.c. in a volumetric flask. Titrate this sugar solution according to directions given under Fehling's Method, page 345.

Calculation.—Make the calculation according to directions given under Fehling's Method, p. 345, bearing in mind that 10 c.c. of Fehling's solution is completely reduced by 0.0676 gram of lactose.

(b) Quantitative Analysis of Gastric Juice.

Töpfer's Method.

This method is much less elaborate than many others but is sufficiently accurate for ordinary clinical purposes. The method embraces the volumetric determination of (1) *total acidity*, (2) *combined acidity*, and (3) *free acidity*, and the subsequent calculation of (4) *acidity due to organic acids and acid salts*, from the data thus obtained.

Strain the gastric contents and introduce 10 c.c. of the strained material into each of three small beakers or porcelain

¹ Almén's reagent may be prepared by dissolving 5 grams of tannin in 240 c.c. of 50 per cent alcohol and adding 10 c.c. of 25 per cent acetic acid.

dishes.¹ Label the vessels *A*, *B* and *C*, respectively, and proceed with the analysis according to the directions given below.

1. **Total Acidity.**²—Add 3 drops of a 1 per cent alcoholic solution of phenolphthalein³ to the contents of vessel *A* and titrate with $\frac{N}{10}$ sodium hydroxide solution until a *dark pink* color is produced which cannot be deepened by further addition of a drop of $\frac{N}{10}$ sodium hydroxide. Take the buret reading and calculate the total acidity.

Calculation.—The total acidity may be expressed in the following ways:

1. The number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide solution necessary to neutralize 100 c.c. of gastric juice.
2. The weight (in grams) of sodium hydroxide necessary to neutralize 100 c.c. of gastric juice.
3. The weight (in grams) of hydrochloric acid which is equivalent to the total acidity of 100 c.c. of gastric juice represents, *i. e.*, percentage of HCl.

The forms of expression most frequently employed are 2 and 3, preference being given to the former.

In making the calculation note the number of cubic centimeters required to neutralize 10 c.c. of the gastric juice and multiply it by 10 to obtain the number of cubic centimeters necessary to neutralize 100 c.c. of the fluid. If it is desired to express the acidity of 100 c.c. of gastric juice in terms of hydrochloric acid, by weight, multiply the value just obtained by 0.00365.⁴

2. **Combined Acidity.**⁵—Add 3 drops of sodium alizarin sulphonate solution⁶ to the contents of vessel *B* and titrate with $\frac{N}{10}$ sodium hydroxide solution until a *violet* color is produced. In this titration the red color, which appears after

¹ If sufficient gastric juice is not available it may be diluted with water or a smaller amount, *e. g.*, 5 c.c., taken for each determination.

² This includes free and combined acid and acid salts.

³ One gram of phenolphthalein dissolved in 100 c.c. of 95 per cent alcohol.

⁴ One c.c. of $\frac{N}{10}$ hydrochloric acid contains 0.00365 gram of hydrochloric acid.

⁵ Hydrochloric acid combined with proteid material.

⁶ One gram of sodium alizarin sulphonate dissolved in 100 c.c. of water.

the tinge of yellow due to the addition of the indicator has disappeared, must be entirely replaced by a *distinct violet color*. Take the burette reading and calculate the combined acidity.

Calculation.—Since the indicator used reacts to all acidities except combined acidity, in order to determine the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide necessary to neutralize the combined acidity of 10 c.c. of the gastric juice, we must subtract the burette reading just obtained from the burette reading obtained in the determination of the total acidity. The data for 100 c.c. of gastric juice may be calculated according to the directions given under Total Acidity, page 384.

3. **Free Acidity.**¹—Add 4 drops of di-methyl-amino-azobenzene (Töpfer's reagent) solution² to the contents of the vessel C and titrate with $\frac{N}{10}$ sodium hydroxide solution until the initial red color is replaced by lemon yellow.³ Take the burette reading and calculate the free acidity.

Calculation.—The indicator used reacts only to free acidity, hence the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide used indicates the volume necessary to neutralize the *free acidity* of 10 c.c. of gastric juice. To determine the data for 100 c.c. of gastric juice proceed according to the directions given under Total Acidity, page 384.

4. **Acidity due to Organic Acids and Acid Salts.**—This value may be conveniently calculated by subtracting the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide used in neutralizing the contents of vessel C from the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide solution used in neutralizing the contents of vessel B. The remainder indicates the number of cubic centimeters of $\frac{N}{10}$ sodium hydroxide solution necessary to neutralize the acidity due to organic acids and acid salts present in 10 c.c. of gastric juice. The data for

¹Hydrochloric acid *not* combined with proteid material.

²One-half gram dissolved in 100 c.c. of 95 per cent alcohol.

³If the lemon yellow color appears as soon as the indicator is added it denotes the *absence* of free acid.

100 c.c. of gastric juice may be calculated according to the methods given under Total Acidity, page 384.

(c) Quantitative Analysis of Blood.

For the methods involved in the quantitative examination of blood see Chapter XI.

APPENDIX.

Almén's Reagent.¹—Dissolve 5 grams of tannin in 240 c.c. of 50 per cent alcohol and add 10 c.c. of 25 per cent acetic acid.

Ammoniacal Silver Solution.²—Dissolve 26 grams of silver nitrate in about 500 c.c. of water, add enough ammonium hydroxide to redissolve the precipitate which forms upon the first addition of the ammonium hydroxide and make the volume of the mixture up to 1 liter with water.

Arnold-Lipliawsky Reagent.³—This reagent consists of two definite solutions which are ordinarily preserved separately and mixed just before using. The two solutions are prepared as follows:

(a) One per cent aqueous solution of potassium nitrite.

(b) One gram of *p*-amino-acetophenon dissolved in 100 c.c. of distilled water and enough hydrochloric acid (about 2 c.c.) added, drop by drop, to cause the solution, which is at first yellow, to become entirely colorless. An excess of acid must be avoided.

Barfoed's Solution.⁴—Dissolve 4 grams of cupric acetate in 100 c.c. of water and acidify with acetic acid.

Baryta Mixture.⁵—A mixture consisting of one volume of a saturated solution of barium nitrate and two volumes of a saturated solution of barium hydroxide.

Boas' Reagent.⁶—Dissolve 5 grams of resorcin and 3 grams of saccharose in 100 c.c. of 95 per cent alcohol.

Congo Red.⁷—Dissolve 0.5 gram of congo red in 90 c.c. of water and add 10 c.c. of 95 per cent alcohol.

¹ Ott's precipitation test, p. 297. Determination of lactalbumin, p. 383.

² Salkowski's method, page 377.

³ Arnold-Lipliawsky reaction, page 308.

⁴ Barfoed's test, page 11.

⁵ Isolation of urea from urine, page 242.

⁶ Test for free acid, page 88.

⁷ Test for free acid, page 88.

Ehrlich's Diazo Reagent.¹—Two separate solutions should be prepared and mixed in definite proportions when needed for use.

(a) Five grams of sodium nitrite dissolved in 1 liter of distilled water.

(b) Five grams of sulphanilic acid and 50 c.c. of hydrochloric acid in 1 liter of distilled water.

Solutions *a* and *b* should be preserved in well stoppered vessels and mixed in the proportion 1:50 when required. Green asserts that greater delicacy is secured by mixing the solution in the proportion 1:100. The sodium nitrite deteriorates upon standing and becomes unfit for use in the course of a few weeks.

Esbach's Reagent.²—Dissolve 10 grams of picric acid and 20 grams of citric acid in 1 liter of water.

Fehling's Solution.³—Fehling's solution is composed of two definite solutions—a cupric sulphate solution and an alkaline tartrate solution, which may be prepared as follows:

Cupric sulphate solution = 34.64 grams of cupric sulphate dissolved in water and made up to 500 c.c.

Alkaline tartrate solution = 125 grams of potassium hydroxide and 173 grams of Rochelle salt dissolved in water and made up to 500 c.c.

These solutions should be preserved separately in rubber-stoppered bottles and mixed in equal volumes when needed for use. This is done to prevent deterioration.

Ferric Alum Solution.⁴—A cold saturated solution.

Folin-Shaffer Reagent.⁵—This reagent consists of 500 grams of ammonium sulphate, 5 grams of uranium acetate and 60 c.c. of 10 per cent acetic acid in 650 c.c. of distilled water.

Furfural Solution.⁶—Add 1 c.c. of furfural to 1000 c.c. of distilled water.

¹ Ehrlich's diazo reaction, page 316.

² Esbach's method, page 344.

³ Fehling's method, page 345. Fehling's test, pages 8 and 286.

⁴ Volhard-Arnold method, page 372.

⁵ Folin-Shaffer method, page 349.

⁶ Mylius's modification of Pettenkofer's test, pages 122 and 301. v. Udránsky's test, pages 123 and 302.

Gallic Acid Solution.¹—A saturated alcoholic solution.

Guaiac Solution.²—Dissolve 0.5 gram of guaiac resin in 30 c.c. of 95 per cent alcohol.

Günzberg's Reagent.³—Dissolve 2 grams of phloroglucin and 1 gram of vanillin in 100 c.c. of 95 per cent alcohol.

Hammarsten's Reagent.⁴—Mix 1 volume of 25 per cent nitric acid and 19 volumes of 25 per cent hydrochloric acid and add 1 volume of this acid mixture to 4 volumes of 95 per cent alcohol. It is preferable that the acid mixture be prepared in advance and allowed to stand until yellow in color before adding it to the alcohol.

Hopkins-Cole Reagent.⁵—To one liter of a saturated solution of oxalic acid add 60 grams of sodium amalgam and allow the mixture to stand until the evolution of gas ceases. Filter and dilute with 2–3 volumes of water.

Hypobromite Solution.⁶—The ingredients of this solution should be prepared in the form of *two* separate solutions which may be united as needed.

(a) Dissolve 125 grams of sodium bromide in water, add 125 grams of bromine and make the total volume of the solution 1 liter.

(b) A solution of sodium hydroxide having a specific gravity of 1.25. This is approximately a 22.5 per cent solution.

Preserve both solutions in rubber-stoppered bottles and when needed for use mix equal volumes of solution *a*, solution *b*, and water.

Iodine Solution.⁷—Prepare a 2 per cent solution of potassium iodide and add sufficient iodine to color it a deep yellow.

Jolles' Reagent.⁸—This reagent has the following composition:

¹ Gallic acid test, page 195.

² Guaiac test, pages 163, 191 and 331.

³ Test for free acid, page 88.

⁴ Hammarsten's reaction, pages 121 and 300.

⁵ Hopkins-Cole reaction, page 45.

⁶ Methods for determination of urea, page 351.

⁷ Iodine test, page 24.

⁸ Jolles' reaction, pages 48 and 292.

Succinic acid	40 grams.
Mercuric chloride	20 grams.
Sodium chloride	20 grams.
Distilled water	1000 gram.s.

Lugol's Solution.¹—Dissolve 5 grams of iodine and 10 grams of potassium iodide in 100 c.c. of distilled water.

Magnesia Mixture.²—Dissolve 175 grams of magnesium sulphate and 350 grams of ammonium chloride in 1400 c.c. of distilled water. Add 700 grams of concentrated ammonium hydroxide, mix thoroughly and preserve the mixture in a glass-stoppered bottle.

Millon's Reagent.³—Digest 1 part (by weight) of mercury with 2 parts (by weight) of HNO_3 (sp. gr. 1.42) and dilute the resulting solution with 2 volumes of water.

Molybdic Solution.⁴—Molybdic solution is prepared as follows, the parts being by weight:

Molybdic acid	1 part.
Ammonium hydroxide (sp. gr. 0.96)	4 parts.
Nitric acid (sp. gr. 1.2)	15 parts.

Mörner's Reagent⁵—Thoroughly mix 1 volume of formalin, 45 volumes of distilled water and 55 volumes of concentrated sulphuric acid.

Neutral Olive Oil.⁶—Shake ordinary olive oil with a 10 per cent solution of sodium carbonate, extract the mixture with ether and remove the ether by evaporation. The residue is *neutral* olive oil.

Nylander's Reagent⁷—Digest 2 grams of bismuth subnitrate and 4 grams of Rochelle salt in 100 c.c. of a 10 per

¹ Gunning's iodoform test, page 304.

² Sodium hydroxide and potassium nitrate fusion method for determination of total phosphorus, page 368.

³ Millon's reaction, page 44.

⁴ Sodium hydroxide and potassium nitrate fusion method for determination of total phosphorus, page 368.

⁵ Mörner's test, page 82.

⁶ Emulsification of fats, page 101.

⁷ Nylander's test, pages 9 and 288.

cent solution of potassium hydroxide. The reagent should then be cooled and filtered.

Obermayer's Reagent.¹—Add 2–4 grams of ferric chloride to a liter of hydrochloric acid (sp. gr. 1.19).

Oxalated Plasma.²—Allow arterial blood to run into an equal volume of 0.2 per cent ammonium oxalate solution.

Paraphenelenediamine Hydrochloride Solution.³—Two grams dissolved in 100 c.c. of water.

Phenolphthalein.⁴—Dissolve 1 gram of phenolphthalein in 100 c.c. of 95 per cent alcohol.

Phenylhydrazin Mixture.⁵—This mixture is prepared by combining 1 part of phenylhydrazin-hydrochloride and 2 parts of sodium acetate *by weight*. These are thoroughly mixed in a mortar.

Phenylhydrazin-Acetate Solution.⁶—This solution is prepared by mixing 1 volume of glacial acetic acid, 1 volume of water and 2 volumes of phenylhydrazin (the base).

Purdy's Solution.⁷—Purdy's solution has the following composition:

Cupric sulphate	4.752	grams.
Potassium hydroxide	23.5	grams.
Ammonia (U. S. P., sp. gr. 0.9)	350.0	c.c.
Glycerin	38.0	c.c.
Distilled water, to make total volume 1 liter.		

Roberts' Reagent.⁸—Mix 1 volume of concentrated nitric acid and 5 volumes of a saturated solution of magnesium sulphate.

Salted Plasma.⁹—Allow arterial blood to run into an equal volume of a saturated solution of sodium sulphate or a 10

¹ Obermayer's test, page 255.

² Experiments on blood plasma, page 167.

³ Detection of hydrogen peroxide, page 196.

⁴ Töpfer's method, page 383.

⁵ Phenylhydrazin reaction, pages 5 and 283.

⁶ Phenylhydrazin reaction, pages 5 and 284.

⁷ Purdy's method, page 347.

⁸ Robert's ring test, pages 48 and 291.

⁹ Experiments on blood plasma, page 167.

per cent solution of sodium chloride. Keep the mixture in the cold room for about 24 hours.

Schweitzer's Reagent.¹—Add potassium hydroxide to a solution of cupric sulphate which contains some ammonium chloride. Filter off the precipitate of cupric hydroxide, wash it and bring it into solution in 20 per cent ammonium hydroxide.

Sherrington's Solution.²—This solution possesses the following formula:

Methylene-blue	0.1 gram.
Sodium chloride	1.2 gram.
Neutral potassium oxalate	1.2 gram.
Distilled water	300.0 grams.

Sodium Acetate Solution.³—Dissolve 100 grams of sodium acetate in 800 c.c. of distilled water, add 100 c.c. of 30 per cent acetic acid to the solution and make the volume of the mixture up to 1 liter with distilled water.

Sodium Alizarin Sulphonate.⁴—Dissolve 1 gram of sodium alizarin sulphonate in 100 c.c. of water.

Solera's Test Paper.⁵—Saturate a good quality of filter paper with 0.5 per cent starch paste containing a little iodic acid and allow the paper to dry in the air. Cut it in strips of suitable size and preserve for use.

Spiegler's Reagent.⁶—This reagent has the following composition:

Tartaric acid	20 grams.
Mercuric chloride	40 grams.
Glycerin	100 grams.
Distilled water	1000 grams.

Standard Ammonium Sulphocyanide Solution.⁷—This solution is made of such a strength that 1 c.c. of it is equa

¹ Schweitzer's solubility test, page 29.

² "Blood counting," page 181.

³ Uranium acetate method, page 367.

⁴ Töpfer's method, page 383.

⁵ Solera's reaction, page 38.

⁶ Spiegler's ring test, pages 48 and 291.

⁷ Volhard-Arnold method, page 372.

to 1 c.c. of the standard argentic nitrate solution mentioned below. To prepare the solution dissolve 12.9 grams of ammonium sulphocyanide, NH_4SCN , in a little less than a liter of water. In a small flask place 20 c.c. of the standard argentic nitrate solution, 5 c.c. of a cold saturated solution of ferric alum and 4 c.c. of nitric acid (sp. gr. 1.2), add water to make the total volume 100 c.c. and thoroughly mix the contents of the flask. Now run in the ammonium sulphocyanide solution from a burette until a permanent *broken* tinge is produced. This is the end-reaction and indicates that the last trace of argentic nitrate has been precipitated. Take the burette reading and calculate the amount of water necessary to use in diluting the ammonium sulphocyanide in order that 10 c.c. of this solution may be exactly equal to 10 c.c. of the argentic nitrate solution. Make the dilution and titrate again to be certain that the solution is of the proper strength.

Standard Argentic Nitrate Solution.¹—Dissolve 29.06 grams of argentic nitrate in 1 liter of distilled water. Each cubic centimeter of this solution is equivalent to 0.01 gram of sodium chloride or to 0.006 gram of chlorine.

Standard Uranium Acetate Solution.²—Dissolve 35.461 grams of uranium acetate in 1 liter of water. One c.c. of such a solution should be equivalent to 0.005 gram of P_2O_5 , phosphoric anhydride.

This solution may be standardized as follows: To 50 c.c. of a standard solution of disodium hydrogen phosphate, of such a strength that the 50 c.c. contains 0.1 gram of P_2O_5 , add 5 c.c. of the sodium acetate solution mentioned on p. 392 and titrate with the uranium solution to the correct end-reaction as indicated in the method proper on p. 367. Inasmuch as 1 c.c. of the uranium solution should precipitate 0.005 gram of P_2O_5 , exactly 20 c.c. of the uranium solution should be required to precipitate the 50 c.c. of the standard phosphate solution. If

¹ Volhard-Arnold method, page 372. Mohr's method, page 371.

² Uranium acetate method, page 367.

the two solutions do not bear this relation to each other the must be brought into the proper relation by diluting the uranium solution with distilled water or by increasing its strength.

Starch Iodide Solution.¹—Mix 0.1 gram of starch powder with cold water in a mortar and pour the suspended starch granules into 75-100 c.c. of boiling water, stirring continuously. Cool the starch paste, add 20-25 grams of potassium iodide and dilute the mixture to 250 c.c. This solution deteriorates upon standing, and therefore must be freshly prepared as needed.

Starch Paste.²—Grind 2 grams of starch powder in a mortar with a small amount of water. Bring 200 c.c. of water to the boiling-point and add the starch mixture from the mortar with continuous stirring. Bring again to the boiling-point and allow it to cool. This makes an approximate 1 per cent starch paste which is a very satisfactory strength for general use.

Stokes' Reagent.³—A solution containing 2 per cent ferrous sulphate and 3 per cent tartaric acid. When needed for use a small amount should be placed in a test-tube and ammonium hydroxide added until the precipitate which forms on the first addition of the hydroxide has entirely dissolved. It produces *ammonium ferrotartrate* which is a reducing agent.

Tanret's Reagent.⁴—Dissolve 1.35 grams of mercuric chloride in 25 c.c. of water, add to this solution 3.32 grams potassium iodide dissolved in 25 c.c. of water, then make the total solution up to 60 c.c. with distilled water and add 20 c.c. of glacial acetic acid to the mixture.

Tincture of Iodine.⁵—Dissolve 70 grams of iodine and 10 grams of potassium iodide in 1 liter of 95 per cent alcohol.

Toison's Solution.⁶—This solution has the following formula:

¹ Fehling's method, page 345.

² Experiments on starch, page 24.

³ Hemoglobin, page 170. Hemochromogen, page 173.

⁴ Tanret's test, pages 48 and 293.

⁵ Smith's test, pages 122 and 301.

⁶ "Blood counting," page 181.

Methyl violet	0.025	gram.
Sodium chloride	1.0	gram.
Sodium sulphate	8.0	grams.
Glycerin	30.0	grams.
Distilled water	160.0	grams.

Töpfer's Reagent.¹—Dissolve 0.5 grams of di-methyl-amino-azobenzene in 100 c.c. of 95 per cent alcohol.

Tropæolin OO.²—Dissolve 0.05 gram of tropæolin OO in 100 c.c. of 50 per cent alcohol.

Uffelmann's Reagent.³—Add a 5 per cent solution of ferric chloride to a 1 per cent solution of carbolic acid until an amethyst-blue color is obtained.

¹ Töpfer's method, page 383.

² Test for free acid, page 89.

³ Uffelman's reaction, page 94.



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